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STANDARDIZATION OF DUST EXTRACTS
I. Standardization on the Basis of Equal Molecular Size

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THERE is need for a certification service for allergenic extracts, comparable to certification of biological stains, that will assure the allergist of the potency and efficacy of the extracts which he purchases and uses for diagnostic and therapeutic purposes. Certification, however, is possible only if a reliable and dependable method of standardizing (or assaying) allergenic extracts is available. The methods that have been in use have been largely chemical, and they have not proven to be dependable or universally acceptable.

Recently, Rockwell, Thomas and Wittich (1947) introduced a method of standardizing house dust extracts which, on the basis of their claims, appeared to offer a satisfactory criterion for certifying such extracts. It seemed worth while, therefore, to investigate the possibility of applying their method to the certification of house dust extracts. Samples of dust extracts prepared according to their directions were, therefore, subjected to analysis and standardization by their method.

EXPERIMENTAL

Preparation of House Dust Extracts.—The samples of house dust used in this investigation were obtained from the vacuum sweepers from local theaters and hotels. A total of four individual batches of dust were obtained from three different sources. Batch 1 was obtained from the vacuum sweeper bag from a local theatre, following a routine rug sweeping. Batches 2, 3, and 4 were obtained from two local hotels which were requested to save the sweepings not only from the rugs but also from the furniture, mattresses, and drapes. In the preparation of both the crude

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TABLE I. RESULTS OF CHEMICAL ANALYSES OF DUST EXTRACTS

Extract	Mg. total N. per ml.	Mg. P.T.A.N.* per ml.	Mg. F.A.A.N.** per ml.
M.R.	0.31450***	0.14225	0.0451
B.B.	1.20540	0.64015	0.1207
M.S.	1.31040	0.59050	0.1372
J.H.	0.67720	0.18760	0.0807
M.H.	1.50080	0.46760	0.0720
R.W.	0.9940	0.19810	0.07466

*Phosphotungstic acid precipitable nitrogen.
**Free alpha amino nitrogen determinations were made on the phosphotungstic precipitate fractions.
***Each value is the average of the results of triplicate determinations.

concentrated and the absorbed concentrated extracts from these batches of dust, the method reported by Rockwell et al was employed.

The crude concentrated extracts were labelled M.R. (from batch 1), B.B. (from batch 2), M.S. (from batch 3), and M.H. (from batch 4.) The absorbed concentrated extracts were labelled J.H. (prepared from M.S.) and R.W. (prepared from M.H.).

Following their preparation, all the dust extracts were filtered through Seitz filters and the filtrates were tested for sterility. Upon confirmation of sterility a portion of each extract was removed and saved for chemical analysis. To the remainder of the extract a volume of sterile glycerin was added to make a 50 per cent solution. All the extracts were stored in sterile rubber-stoppered, heavy pyrex 250 ml. Erlenmeyer flasks at 8° C.

Chemical Standardization.—The procedures used for assaying the potency of the dust extract samples were those used by Rockwell et al (1947). Accordingly, total nitrogen, phosphotungstic acid precipitable nitrogen, and free alpha amino nitrogen determinations were made. The micro Kjeldahl procedure used for the total nitrogen and phosphotungstic acid precipitable nitrogen determinations was that of Parnas and Wagner (1931). The free alpha amino nitrogen determinations were made according to the method of Peters and Van Slyke (1932).

For all three determinations (total nitrogen, phosphotungstic acid precipitable nitrogen, and free alpha amino nitrogen) each sample was run in triplicate, and at the start of each series of runs a blank determination was made on the reagents alone.

Table I shows the results of the chemical examinations of each extract. Each value represents the average of the results of the triplicate analyses.

Standardization of the House Dust Extracts by Intradermal Testing.—

1. Comparison of skin reactions of dust extracts not diluted to a constant P.T.A. nitrogen concentration:

Each of the six extracts was dispensed, aseptically, in 10 ml. amounts, in diaphragm-stoppered vials. A set of vials was sent to each of five cooperating allergists for skin testing. The extracts were accompanied by record sheets upon which the results were to be recorded, together with other data requested (Fig. 1) and a copy of instructions.

Test done by: Dr. Return to: Dr. M. Scherago
Dept. of Bact.
Univ. of Ky.
Lexington, Ky.

Patient: Clinical notations:

	Dilutions 1:10	1:100	1:1,000	1:10,000
Extract No. Amount Injected Date				
Extract No. Amount Injected Date				
Extract No. Amount Injected Date				

Fig. 1. Sample record sheet for recording skin test reactions produced by dust extracts that had not been diluted to a constant P.T.A. nitrogen content.

INSTRUCTION SHEET

1. All tests are to be made intradermally.
2. Please keep antigens in refrigerator when not being used.
3. Use properly cleaned and sterilized sharp 26-gauge hypodermic needles and tuberculin syringes.
4. The amount injected should be 0.02 c.c., accurately measured for all tests and recorded on the data sheet.
5. The dilutions to be used are prepared with physiological saline (0.85 per cent NaCl). For dust-sensitive patients make 1:10, 1:100, 1:1,000, 1:10,000 dilutions of each sample. For non-sensitive controls use only the 1:10, and 1:100 dilutions.
6. Each patient is to be tested with all six samples in the above dilutions.
7. If possible, test at least three known dust-sensitive patients with the samples in all dilutions and one non-sensitive patient as a control.
8. Injections: Make injections approximately two inches apart with the least possible trauma in the volar surface of the forearm and lateral surface of the arm. Injections are preferably made by the same individual so that the technique will be about the same.
9. Reactions: Skin reactions may be read with a 75 watt Mazda lamp or by daylight. Observations of the skin reactions should be made at fifteen minutes and readings taken about twenty minutes after the injection. When the wheals and flares are of their maximum size they should be recorded by *one* of two methods:

(a) Tracings: The wheals and flares are outlined on the skin with a washable ink. Thin tissue paper is placed over them and both the wheal formation area and erythema are traced in pencil. These tracings should then be transferred by means of carbon paper to the enclosed data sheet.

(b) Measurements: The wheal and flare should be measured in millimeters and recorded as $\frac{\text{wheal}}{\text{flare}}$, for example a wheal measuring 1 by 1 mm. with a flare of 2 by 4 mm. should be recorded as $\frac{1 \times 1}{2 \times 4}$. A notation of "ps" should be made if pseudopodia develop; thus if there were pseudopodia it would be recorded as $\frac{1 \times 1}{2 \times 4}$ ps.

TABLE II. SKIN REACTIONS TO HOUSE DUST EXTRACTS NOT DILUTED TO THE SAME P.T.A. NITROGEN CONTENT

Allergist:		Kaplan		Rockwell		Stier				Mothersi				
Patient:		A.D.	F.G.	R.	H.S.	M.H.	D.M.	F.B.	P.Mc.	S.T.	R.W.	A.B.	R.M.	M.E.
Dust Extract	Dilution													
M.R.	1-10	9x5**	0x0	13x12	15x12	8x8	10x8	12x12	7x7	10x8	9x9	*	10x10	10x12
		19x13	0x0	33x32	30x30	25x30	15x15	20x25	15x15	20x15	20x40		30x0	30x30
	1-100	3x4	0x0	12x9	12x10	7x7	6x6	12x10	6x6	10x12	5x5	6x6	7x8	5x5
		7x6	0x0	30x30	30x25	20x20	15x15	30x30	15x15	20x22	0x0	15x16	0x0	20x50
	1-1000	0x0	0x0	5x5	9x7	6x6	0x0	10x8	6x6	0x0	10x15	7x8	0x0	5x5
		0x0	0x0	24x26	30x20	15x15	0x0	20x20	10x10	0x0	25x20	20x30	0x0	20x20
	1-10000	0x0	0x0	4x4	8x6	5x5	0x0	7x8	5x5	0x0	2x3	3x2	*	5x5
		0x0	0x0	24x24	20x20	10x10	0x0	20x15	10x10	0x0	0x0	10x5		0x0
B.B.	1-10	4x3	5x4	17x14	15x20	10x10	8x8	9x10	15x10	12x10	10x10	*	10x12	0x0
		16x19	26x18	42x40	30x30	25x25	10x5	30x20	25x25	35x35	20x30		0x0	0x0
	1-100	3x3	9x6	13x10	12x12	8x8	8x8	8x9	10x12	12x14	5x5	10x8	10x8	10x10
		12x9	26x18	30x33	25x25	20x20	15x15	20x20	20x20	25x26	15x10	20x20	20x20	20x30
	1-1000	0x0	6x4	9x10	10x8	6x6	8x8	8x8	10x12	10x10	10x10	10x12	0x0	0x0
		0x0	0x0	21x22	25x20	15x15	15x15	20x15	20x20	12x10	20x20	30x35	0x0	0x0
	1-10000	0x0	6x3	3x4	8x6	6x6	8x8	6x6	7x6	0x0	7x2	5x5	*	0x0
		0x0	0x0	14x12	20x20	10x10	15x15	20x15	15x15	0x0	0x0	25x20		0x0
M.S.	1-10	3x3	0x0	18x16	14x12	8x8	8x8	8x8	10x12	20x10	10x10	*	12x13	15x10
		9x9	0x0	38x37	40x30	20x20	15x15	25x20	25x25	25x32	20x30		20x30	20x30
	1-100	0x0	0x0	10x14	12x10	8x6	8x8	8x8	8x6	20x18	5x5	15x10	2x3	5x5
		0x0	0x0	22x22	30x30	15x15	15x15	20x20	15x15	25x20	20x30	22x25	0x0	10x20
	1-1000	0x0	0x0	8x8	8x10	0x0	8x8	6x8	6x6	5x5	10x10	10x10	0x0	0x0
		0x0	0x0	10x12	25x30	0x0	15x15	15x15	10x10	10x10	15x15	0x0	0x0	0x0
	1-10000	0x0	0x0	4x5	8x8	0x0	0x0	5x5	6x5	10x10	10x5	3x3	*	0x0
		0x0	0x0	8x9	25x30	0x0	0x0	10x10	10x10	12x12	0x0	15x10		0x0

*Not tested in that dilution.
**Numerator—diameters of the wheal in millimeters as measured through its two extremes.
Denominator—diameters of the flare in millimeters as measured through its two extremes.

Of the five allergists who had indicated a willingness to co-operate and to whom a set of the extracts to be tested had been sent, four responded with reports. These four allergists tested a total of thirteen house-dust-sensitive patients and six non-house-dust-sensitive controls. No positive reactions were obtained with any of the extracts in the control persons tested. The skin reactions obtained in the dust-sensitive persons are recorded in Table II. They are recorded as the diameters of the wheal in millimeters as measured through its two extremes over the diameters of the flare as measured through its two extremes. As may be seen from this table, there was considerable variation in degree of sensitivity to the six extracts among the patients tested. Nevertheless, the less reactive patients did not react at all to the extracts that produced reactions only in low dilutions in those patients that were more sensitive.

TABLE II. SKIN REACTIONS TO HOUSE DUST EXTRACTS NOT DILUTED TO THE SAME P.T.A. NITROGEN CONTENT—CONTINUED

Allergist:		Kaplan		Rockwell		Stier				Mothersill				
Patient:		A.D.	F.G.	R.	H.S.	M.H.	D.M.	F.B.	P.Mc.	S.T.	R.W.	A.B.	R.M.	M.E.
Dust Extract	Dilution													
M.H.	1-10	5x6	5x4	14x16	15x12	12x12	10x8	10x8	10x10	15x10	7x7	*	10x13	13x12
		35x38	15x14	32x32	30x30	30x25	30x30	25x25	20x20	30x30	20x40		0x0	30x20
	1-100	0x0	6x5	12x12	12x15	10x10	8x8	8x8	10x8	10x15	20x25	10x10	4x5	10x12
		0x0	23x14	29x30	30x20	15x15	15x15	15x15	15x15	30x30	26x30	20x25	20x30	20x25
	1-1000	0x0	4x5	8x7	10x8	8x8	0x0	8x8	8x6	10x10	9x9	10x12	0x0	0x0
		0x0	13x8	25x23	25x25	10x10	0x0	15x10	15x15	25x25	20x20	30x40	0x0	0x0
	1-10000	0x0	9x5	5x4	8x8	8x8	0x0	6x6	6x6	7x7	4x3	0x0	*	5x5
		0x0	17x10	15x16	20x25	10x10	0x0	10x10	10x10	20x20	20x15	0x0		0x0
J.H.	1-10	3x3	6x3	8x7	12x10	0x0	0x0	8x10	10x8	8x6	4x5	*	8x7	0x0
		9x9	14x9	36x35	25x20	0x0	0x0	20x25	20x20	14x10	5x6		0x0	0x0
	1-100	0x0	4x2	9x5	10x10	0x0	0x0	8x8	8x8	5x5	5x5	6x5	5x4	0x0
		0x0	14x9	30x35	20x15	0x0	0x0	15x20	15x15	0x0	0x0	7x6	0x0	0x0
	1-1000	0x0	3x3	4x3	8x8	0x0	0x0	6x6	6x6	0x0	0x0	3x4	0x0	0x0
		0x0	12x11	14x12	20x20	0x0	0x0	15x15	15x15	0x0	0x0	15x15	0x0	0x0
	1-10000	0x0	0x0	0x0	6x8	0x0	0x0	6x6	5x5	0x0	0x0	5x5	*	0x0
		0x0	0x0	0x0	20x15	0x0	0x0	10x10	10x10	0x0	0x0	20x20		0x0
R.W.	1-10	0x0	5x4	0x0	8x8	0x0	0x0	8x8	8x8	5x5	0x0	*	0x0	6x7
		0x0	25x16	0x0	20x15	0x0	0x0	15x15	15x15	0x0	0x0		0x0	0x0
	1-100	0x0	3x3	0x0	8x8	0x0	0x0	8x8	8x8	6x5	0x0	5x5	0x0	0x0
		0x0	16x11	0x0	10x10	0x0	0x0	15x15	15x15	10x10	0x0	0x0	0x0	0x0
	1-1000	0x0	3x2	0x0	8x8	0x0	0x0	8x6	8x6	0x0	0x0	5x5	0x0	0x0
		0x0	19x15	0x0	10x10	0x0	0x0	15x15	15x15	0x0	0x0	15x15	0x0	0x0
	1-10000	0x0	0x0	0x0	6x6	0x0	0x0	8x8	6x6	0x0	0x0	5x5	*	0x0
		0x0	0x0	0x0	15x10	0x0	0x0	15x15	15x15	0x0	0x0	0x0		0x0

*Not tested in that dilution.
**Numerator—diameters of the wheal in millimeters as measured through its two extremes.
Denominator—diameters of the flare in millimeters as measured through its two extremes.

In an effort to evaluate the potencies of the extracts, an evaluation code, consisting of two groups of digits, was employed. The first group, consisting of a single digit on the left, was used to indicate the highest dilution giving a reaction of at least 5 mm. by 5 mm. in size.

1 equals 1-10	3 equals 1-1000
2 equals 1-100	4 equals 1-10000

The second group, consisting of the remaining digits, was used to indicate the measurements of the wheal in millimeters in that dilution. The results of the skin reactions according to this code are recorded in Table III.

The results as recorded in this table were then averaged for each of the six extracts in the following way. The first digits representing the highest

TABLE III. SKIN REACTIVITY RATINGS OF DUST EXTRACTS TESTED ON DUST SENSITIVE PATIENTS

Allergist	Patient	Dust Extracts					
		Crude			Adsorbed		
		M.R.	B.B.	M.S.	M.H.	J.H.	R.W.
Kaplan	A.D.	1—9x5*	1—4x3	1—4x9	1—5x6	1—3x3	neg.
	F.G.	neg.	3—6x4	neg.	4—9x5	1—6x3	1—5x4
Rockwell	R.	3—5x5	3—9x10	3—8x8	3—8x7	2—9x5	neg.
Stier	H.S.	4—8x6	4—8x6	4—8x8	4—8x8	4—6x8	4—6x6
	M.H.	4—5x5	4—6x6	2—8x6	4—8x8	neg.	neg.
	D.M.	2—6x6	4—8x8	3—8x8	2—8x8	neg.	neg.
	F.B.	4—7x8	4—6x6	4—5x5	4—6x6	4—6x6	4—8x8
	P.Mc.	4—5x5	4—7x6	4—6x5	4—6x6	4—5x5	4—6x6
Mothersill	S.T.	2—10x12	3—10x10	4—10x10	4—7x7	2—5x5	2—6x5
	R.W.	3—10x15	3—10x10	4—10x5	3—9x9	2—5x5	neg.
	A.B.	3—7x8	4—5x5	3—10x10	3—10x12	4—5x5	4—5x5
	R.M.	2—7x8	2—10x8	1—12x13	1—10x13	1—8x7	neg.
	M.E.	4—5x5	2—10x10	2—5x5	2—10x12	neg.	1—6x7

*First digit designates the highest dilution giving a reaction 5 mm x 5 mm (1 = 1-10, 2 = 1-100, 3 = 1-1000) the second factor (e.g. 9x5) designates the diameters of the wheal in mm in that dilution.

TABLE IV. COMPARISON OF AVERAGE VALUES OF THE SKIN TEST RATINGS OF THE DUST EXTRACTS WITH THE AVERAGE VALUES OF THE TOTAL NITROGEN AND P.T.A. NITROGEN

Extract	Skin Test Rating	Total N. mg./ml.	P.T.A.N.* mg./ml.
B.B.	3.22	1.2054	0.64015
M.H.	3.16	1.5008	0.4676
M.R.	2.84	0.3145	0.1432
M.S.	2.76	1.3104	0.5905
J.H.	1.97	0.6772	0.1876
R.W.	1.57	0.9940	0.1981

*Phosphotungstic acid precipitable nitrogen.

dilution of extract that gave a positive reaction were averaged to the third decimal. Next the average diameter of the wheal was computed for each extract as tested on each patient, and these figures averaged. The resulting figure for each extract was then added to the last two digits (the second and third decimals) of the figure obtained by averaging the dilutions. In this manner the size of the reaction produced as well as the dilution of the reaction was given consideration. These average values (skin test ratings) are recorded in Table IV. As may be seen from this table, the six extracts, as tested in thirteen different house-dust-sensitive patients, ranged in potency in the following descending order, B.B., M.H., M.R., M.S., J.H., and R.W.

A comparison of the skin reactivity ratings with the results of the chemical analyses of these extracts is also shown in Table IV, and in graphic form in Figure 2. From Table IV and Figure 2, it may be seen that the skin reactivity of each of the six extracts was not a function of either the total nitrogen concentration or the phosphotungstic acid precipitable nitrogen concentration.

2. Comparison of the skin reactivities of dust extracts of approximately the same molecular size and diluted to the same P.T.A. nitrogen concentration:

Rockwell et al (1947) reasoned that since the milligrams per ml. of P.T.A. nitrogen represent the total amount of protein nitrogen present in a sample of house dust extract and the milligrams of free alpha amino

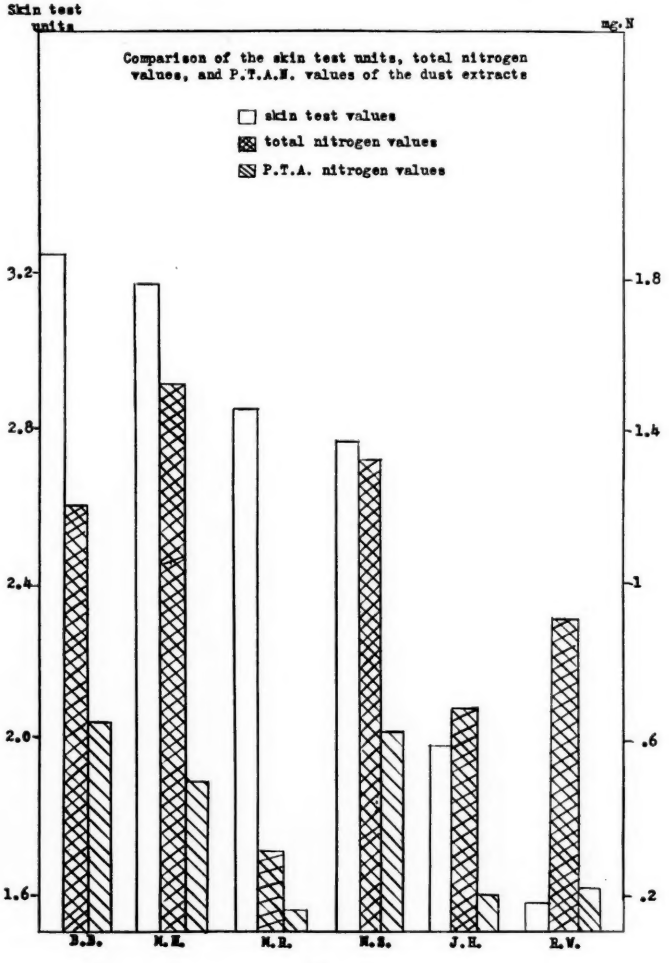

Fig. 2

nitrogen represent the number of free alpha amino groups in the total protein, it is possible to calculate, by dividing the P.T.A. nitrogen by the free alpha amino nitrogen, the average number of nitrogen atoms per molecule of sample. This, they reasoned, was possible since each protein molecule contains only one free alpha amino group. Accordingly, they calculated these values for each of the extracts that they had used. They

then selected extracts which they considered to be of approximately the same molecular size (those that contained from 9 to 14 nitrogen atoms per molecule, or a maximum difference from the smallest to the largest of

Test done by: Dr.	Clinical Notations:	Return to: Dr. M. Scherago Dept. of Bact. Univ. of Ky. Lexington, Ky.
Patient:	Dilutions	
	Concentrated	1:10
Extract No. Amount Injected Date		
Extract No. Amount Injected Date		
Extract No. Amount Injected Date		

Fig. 3. Sample record sheet for recording skin test reactions produced by dust extracts that had been diluted to a constant P.T.A. nitrogen content.

TABLE V. AVERAGE NUMBER OF NITROGEN ATOMS PER MOLECULE OF THE PREPARED EXTRACTS

	Extract	Average Number of Nitrogen Atoms per Molecule
Crude	M.R.	3
	B.B.	5
	M.S.	4
	M.H.	6
Adsorbed	J.H.	2
	R.W.	3

5 atoms). They diluted each of these extracts so as to contain 0.300 mg. of P.T.A. nitrogen per ml. From these diluted extracts they prepared 1-100 and 1-1000 dilutions and mailed them to co-operating allergists with instructions to test the dilutions in both sensitive and nonsensitive persons.

When we calculated the average number of nitrogen atoms per molecule of extract of our extracts, using the values recorded in Table I, we obtained the results recorded in Table V. The figures recorded in this table are the whole numbers most closely approximating the decimal values obtained by dividing the P.T.A. nitrogen by the free alpha amino nitrogen values. Only whole numbers were used because fractions of a nitrogen atom cannot exist. Each of the extracts was diluted to contain 0.003 mg. of P.T.A. nitrogen per ml., which is the amount that was contained in the 1-100 dilution of Rockwell et al. Each extract that was so diluted was dis-

TABLE VI. SKIN REACTIONS TO HOUSE DUST EXTRACTS DILUTED TO THE SAME P.T.A. NITROGEN CONTENT

Allergist	Patient	Extracts											
		Crude						Adsorbed					
		M.R.		B.B.		M.S.		M.H.		J.H.		R.W.	
		Conc.	1-10	Conc.	1-10	Conc.	1-10	Conc.	1-10	Conc.	1-10	Conc.	1-10
Brown	A.F.	13x10	13x10	12x10	8x6	12x13	12x8	12x8	9x7	5x5	8x5	9x6	8x4
		39x41	33x29	24x20	0x0	42x39	0x0	37x33	30x20	0x0	0x0	0x0	0x0
	R.A.	13x14	13x9	10x7	8x10	8x7	10x10	11x9	6x10	7x8	9x12	10x8	10x8
		34x33	23x26	28x33	0x0	24x20	0x0	39x31	0x0	0x0	0x0	28x32	0x0
	P.N.	17x11	13x9	13x11	11x9	15x13	10x7	20x10	8x8	10x8	10x7	10x8	10x8
		42x35	34x31	33x31	32x30	38x39	35x39	37x35	28x25	38x40	40x35	29x28	30x26
	S.G.	11x11	9x8	10x7	8x5	8x6	7x6	11x9	5x6	6x5	7x3	6x6	4x3
		29x32	0x0	30x31	0x0	0x0	0x0	15x16	0x0	0x0	0x0	0x0	0x0
	E.M.	15x8	10x10	12x13	8x8	13x9	11x12	10x8	7x7	6x6	6x5	16x9	8x5
		40x31	0x0	29x32	0x0	31x36	20x20	0x0	0x0	0x0	0x0	27x29	0x0
	V.M.	19x13	17x12	11x12	8x6	11x8	7x6	9x8	7x5	7x6	5x5	8x7	6x7
		40x36	33x38	32x33	29x30	34x28	0x0	28x30	0x0	0x0	0x0	23x24	34x30
	S.A.	12x11	9x9	23x12	8x8	11x7	8x6	6x5	13x10	10x7	7x6	12x11	5x8
		34x34	35x36	44x45	24x21	27x31	24x18	24x24	44x39	0x0	21x21	33x36	17x14
	H.W.	19x16	16x12	15x16	16x15	14x9	8x6	15x11	8x7	12x9	8x6	10x7	12x9
		41x38	30x33	40x36	40x38	37x36	35x36	31x28	25x27	34x35	0x0	34x30	32x30
Hyman	W.R.	13x10	6x8	10x5	10x8	12x9	6x6	9x8	9x6	5x5	7x7	8x8	7x5
		38x33	17x19	32x30	24x27	39x34	15x12	28x31	21x13	10x7	23x13	23x18	17x14
	P.L.	14x12	13x10	14x14	14x12	15x16	9x7	15x11	14x10	8x6	13x10	9x5	8x10
		42x39	39x34	47x41	42x47	39x37	41x31	39x30	45x28	24x15	44x31	32x27	0x0
	E.S.	11x8	12x8	11x11	7x4	9x7	5x5	8x9	6x5	9x4	4x6	6x5	8x5
		34x29	0x0	30x19	0x0	28x26	0x0	24x13	0x0	0x0	0x0	0x0	0x0
	S.	12x12	13x9	12x9	8x6	7x10	11x9	8x11	10x10	7x4	8x8	8x5	5x4
		41x34	33x37	24x21	33x23	34x25	25x21	14x10	30x20	28x30	30x20	27x26	16x13
Davison	P.C.	0x0	5x7	5x4	0x0	0x0	0x0	3x3	0x0	0x0	0x0	0x0	0x0
		14x15	30x40	25x35	0x0	0x0	35x35	30x30	0x0	0x0	0x0	0x0	0x0
	M.H.	10x10	0x0	12x10	4x4	7x6	0x0	9x10	0x0	0x0	0x0	8x6	0x0
		30x45	7x14	29x30	7x7	38x52	6x6	40x63	6x5	5x5	0x0	28x30	0x0
	L.H.	5x5	0x0	5x5	0x0	4x6	0x0	5x5	0x0	0x0	0x0	0x0	0x0
		10x10	0x0	0x0	0x0	6x6	0x0	0x0	0x0	0x0	0x0	0x0	0x0
	M.M.	5x5	0x0	2x2	0x0	0x0	0x0	4x4	3x3	5x5	0x0	0x0	0x0
		0x0	0x0	0x0	0x0	0x0	0x0	0x0	0x0	0x0	0x0	0x0	0x0

Numerator—diameters of the wheal in millimeters as measured through its two extremes.
Denominator—diameters of the flare in millimeters as measured through its two extremes.

TABLE VII. SKIN REACTIVITY RATINGS OF DUST EXTRACTS AFTER DILUTION TO A CONSTANT CONCENTRATION OF P.T.A. NITROGEN

Allergist	Patient	Extracts					
		Crude			Adsorbed		
		M.R.	B.B.	M.S.	M.H.	J.H.	R.W.
Brown	A.F.	3—13x10*	3—8x6	3—12x6	3—9x7	3—8x5	3—8x4
	R.A.	3—13x9	3—8x10	3—10x8	3—6x10	3—9x12	3—10x8
	P.N.	3—13x9	3—11x9	3—10x7	3—8x8	3—7x10	3—10x8
	S.G.	3—9x8	3—8x5	3—7x6	3—5x6	3—7x3	2—6x6
	E.M.	3—10x10	3—8x8	3—11x12	3—7x7	3—6x5	3—8x5
	V.M.	3—17x12	3—8x6	3—7x6	3—7x5	3—5x5	3—6x7
	S.A.	3—9x9	3—8x8	3—8x6	3—10x13	3—7x6	3—5x8
	H.W.	3—16x12	3—16x15	3—8x6	3—8x7	3—8x6	3—12x9
Hyman	W.R.	3—6x8	3—10x8	3—6x6	3—9x6	3—7x7	3—7x5
	P.L.	3—13x10	3—14x12	3—9x7	3—14x10	3—13x10	3—8x10
	E.S.	3—12x8	3—7x4	3—5x5	3—6x5	3—4x6	3—8x5
	S.	3—13x9	3—8x6	3—11x9	3—10x10	3—8x8	2—8x5
Davison	P.C.	3—5x7	2—5x4	0x0	2—3x3	0x0	0x0
	M.H.	2—10x10	2—12x10	2—7x6	2—9x10	0x0	2—8x6
	L.H.	2—5x5	2—5x5	2—4x6	2—5x5	0x0	0x0
	M.M.	2—5x5	2—2x2	0x0	2—4x4	2—5x5	0x0

*First digit designates the highest dilution giving a reaction 5 mm by 5 mm (2 = undiluted; 3 = 1-10). The second factor (e.g. 12x10) designates the diameters of the wheal in millimeters in that dilution.

pensed in diaphragm-stoppered vials and appropriately labelled. A set of samples of all the extracts was mailed to each of five allergists for intradermal testing. Accompanying the extracts were record sheets (Fig. 3) upon which the results were to be recorded and a list of directions identical with those given in the preceding experiments, except for item 5 which was changed to read as follows: "The dilutions to be injected are (1) concentrated, (2) 1-10 dilution of the concentrated prepared with physiological saline (0.85 per cent NaCl)." Thus each allergist was instructed to use the same dilutions employed by Rockwell et al.

Of the five allergists to whom samples were sent for skin testing three responded with reports. The three allergists tested a total of sixteen patients that were sensitive to dust and an additional five patients that were not sensitive. In every case the six extracts failed to produce a reaction when tested in the non-dust-sensitive controls. The results of the skin reactions as tested in the dust-sensitive patients are recorded in Table VI. Here too, the skin reactions are recorded as the diameters of the wheal in mm. measured through its two extremes over the diameters of the flare in mm. measured through its two extremes. The results of the skin tests were condensed and evaluated according to the code given above for that purpose. In accordance with the practice of Rockwell et al, only the size of the wheal was considered in evaluating the potency of the extracts. The values thus obtained are recorded in Table VII. As may be seen from Table VII, there is considerable variation in the degree of sensitivity among the patients. Thus eight patients tested by Dr. Brown seemed to be highly reactive to our samples, in contrast to the four patients tested by Dr. Davison. The four patients tested by Dr. Hyman seemed to be moderately reactive. Despite the variations in the degree of sensitivity between the individual patients the trend establishing the relative order of potency of the six ex-

tracts was consistent. For example, extract M.R. was the most reactive in eleven cases and second most reactive in three additional cases of the sixteen patients that were tested.

TABLE VIII. AVERAGE VALUES OF THE SKIN TEST RATINGS OF THE DUST EXTRACTS AFTER DILUTION TO A CONSTANT P.T.A.N.* CONCENTRATION

	Dust Extract	Average Rating of Skin Tests
Crude	M.R.	2.92
	M.H.	2.83
	B.B.	2.80
Adsorbed	M.S.	2.56
	J.H.	2.46
	R.W.	2.25

*Phosphotungstic acid precipitable nitrogen

The averages of the skin test values, computed in the same way as those in the previous experiment, were then determined for the six extracts. These averages arranged in the order of potency of the extracts are recorded in Table VIII. As may be seen from this table, extracts M.R., M.H., and B.B. (with molecular sizes of 3, 6, and 5, respectively) gave approximately the same skin test ratings. M.S. and J.H. (with molecular sizes of 4 and 2, respectively) also differed very little from each other in their skin test ratings. There was, however, no correlation between the skin test ratings of M.S. and J.H. and those of M.R., M.H., and B.B. and none between the skin test rating of R.W. (molecular size of 3) and that of any of the other extracts. For the six extracts the skin reactivity varied from a value of 2.25 to one of 2.92. A comparison between the averages of the skin test values, as shown in Table VIII and the concentrations of phosphotungstic acid precipitable nitrogen is shown graphically in Figure 4. As may be seen from this figure, while the phosphotungstic acid precipitable nitrogen of the extracts remained constant at 0.003 mg./ml., the skin reactivity varied considerably. These wide variations in potency are in contrast to the findings of Rockwell et al who reported approximately equal skin reactions to the extracts which they had standardized in this manner.

DISCUSSION

In our attempt to apply the method of standardizing dust extracts proposed by Rockwell, Thomas and Wittich (1947) to the certification of such extracts, every effort was made to follow as explicitly as possible the directions which they gave not only for analyzing and standardizing the extracts but for their preparation as well. Prior to the performance of the chemical analyses on the six house dust extracts, numerous preliminary trials were performed on carefully prepared and standardized solutions in order to become as expert as possible in the techniques involved. Standard

solutions of urea were prepared and analyzed by the micro Kjeldahl procedure. These preliminary analyses were carried out until the correct values of nitrogen were consistently obtained. Repeated determinations of

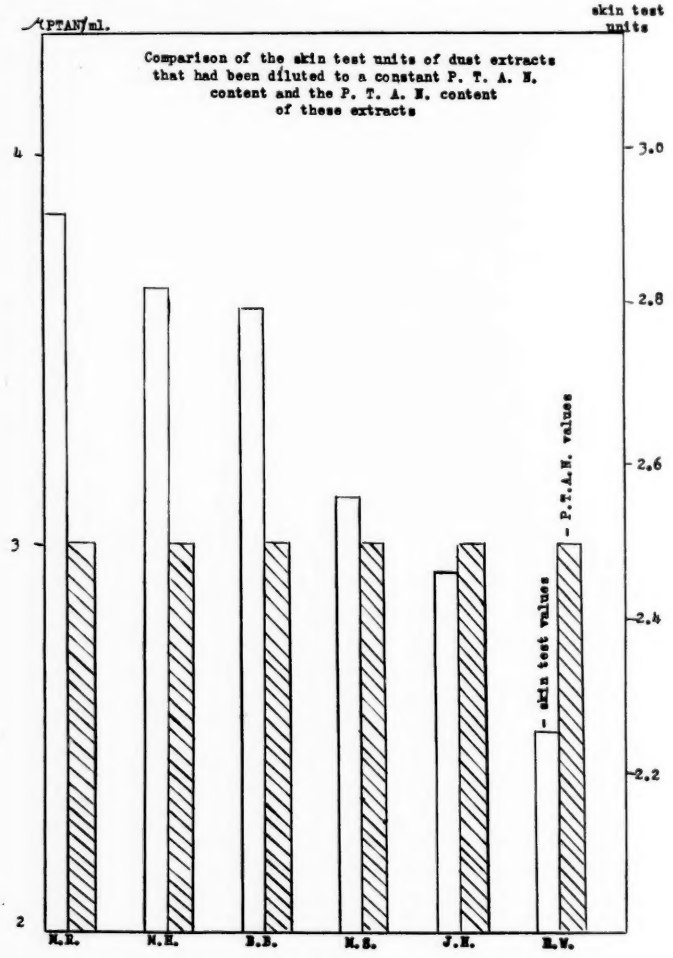

Fig. 4

free alpha amino nitrogen were carried out on prepared samples of glycine by the Van Slyke procedure under the instruction and guidance of a trained and experienced operator of this apparatus until consistently accurate results were obtained. All the tests were carried out by two workers working together and checking each other.

Our failure to obtain any correlation whatsoever between the skin test

activities of the six dust extracts which we prepared and examined and their total or phosphotungstic acid precipitable nitrogen content confirms the findings in this respect of Rockwell et al and of others and substantiates the observations of many workers, including Arbesman and Eagle (1939) and Sutherland (1945), who believe that estimations of nitrogen are not valid criteria for determining allergenic potencies.

When our extracts, which were all of approximately equal molecular size, were each diluted to contain 0.003 mg. per ml. of P.T.A. nitrogen and tested on both sensitive and non-sensitive persons, as was done by Rockwell et al, considerable variation was found in the skin potencies of the extracts. This finding was in marked contrast to that of Rockwell and his colleagues, who found almost perfect agreement in the potencies of the extracts which they studied in this manner.

Because of our failure to confirm the latter finding of Rockwell et al, the data in their report were subjected to a very thorough analysis in the hope that we might find some explanation for the disagreement between their results and ours. This analysis revealed certain discrepancies in their data. According to Table V of their report, extracts AA, RB, UF, WX, MB, and MC, all of approximately equal molecular size, gave, after dilution to a constant protein nitrogen concentration and after injection in only 1-100 and 1-1000 dilutions, skin reactivity ratings above 3.50 in every case, except one, in which the rating was 3.47. From the evaluation key on page 31 of their report the maximum reaction obtainable with a 1-1000 dilution was 3. It was difficult, therefore, to understand how they could have arrived at skin reactivity ratings averaging above 3. Furthermore, in Table IV, these same extracts, with the exception of MC, which were tested before they were diluted to a constant phosphotungstic acid precipitable nitrogen concentration, show no correlation between their skin reactivity ratings and their phosphotungstic acid precipitable nitrogen content. For example, extract WX, which contained 0.3238 mg. of phosphotungstic acid precipitable nitrogen per ml., gave a skin reactivity rating of 5.00, while extract UF, which contained 0.9680 mg. of phosphotungstic acid precipitable nitrogen per ml., gave a skin reactivity rating of only 3.90. It was difficult to understand how two extracts of approximately equal molecular size, one containing three times the phosphotungstic acid precipitable nitrogen concentration as the other, and having a skin reactivity rating of 3.90 as compared to a rating of 5.00 for the other, could produce, after dilution to equal phosphotungstic acid precipitable concentrations, skin reactions of the same magnitude. These discrepancies were, therefore, called to Dr. Rockwell's attention and Dr. Rockwell concurred in our criticism. Dr. Rockwell agreed that there appeared to be some error in the published paper. After he had had an opportunity to compare the published paper with the original manuscript, he wrote that in reference to the skin reactivity ratings of the molarly standardized extracts all averaging above 3.00, the code used to arrive at these values in the

original manuscript was erroneously omitted by the editors of the journal in which the paper was published. The code that was omitted from the published paper was as follows:

Dilution	*Average Diameter of Wheal in Millimeters*	*Rating of Skin Reactivity*
	10 or more	5
1-1000	8 or 9	4
	5, 6, or 7	3
1-100	5 or more	2
	3 or 4	1

With regard to the discrepancy concerning the lack of correlation between the skin test values of the same extract when tested non-molarly and molarly, he stated that again the editors were at fault. All the extracts tested on a molar basis had been purified (no method stated) before they were analyzed chemically and tested for skin potency. The statement in the manuscript regarding the necessity for purifying these extracts before skin testing and the letter S which had been added to the designations of the extracts to distinguish the purified extracts from the crude ones from which they had been derived (e.g. W.X. to W.X.S.) were erroneously omitted from the published paper.

It is unfortunate, of course, that such serious editorial errors should have been made in the published Rockwell report. However, although the corrections listed by Dr. Rockwell would seem to explain satisfactorily the discrepancies in the report, they do not account for our failure to confirm the findings in that report. When we found that the code for evaluating skin reactivity, as published in the report, was not sensitive enough to detect all the variations in our molarly standardized extracts, we adopted an alternative procedure. It was the results of the evaluation of our extracts by this procedure that failed to confirm the findings of Rockwell et al. After Dr. Rockwell revealed that a more sensitive code had been used by him and his colleagues, we applied this code to our data. The results which we obtained on the basis of this code are given in Table IX. As may be seen from this table, even by the use of this code the marked degree of variation between the molarly standardized extracts is clearly revealed.

Since it has not been proved that the active fraction in dust extracts is a protein, it would appear to be unsound to base any chemical assay procedures upon the estimation of nitrogen in any form. However, even assuming that the house dust active component is a protein, it is possible that the amounts of this active protein component in various dust extracts may vary considerably while the total protein content, as estimated by P.T.A. nitrogen, remains constant. It appears obvious, therefore, that a chemical method of standardization that is based upon the estimation of an unknown entity is not well founded.

TABLE IX. SKIN REACTIVITY RATINGS OF PREPARED EXTRACTS DILUTED TO A CONSTANT P.T.A. NITROGEN CONCENTRATION*

Allergist	Patient	Extract					
		Crude			Adsorbed		
		M.R.	B.B.	M.S.	M.H.	J.H.	R.W.
Brown	A.F.	5	3	5	4	3	3
	R.A.	5	4	5	4	5	4
	P.N.	5	5	4	4	4	4
	S.G.	4	3	3	3	3	2
	V.M.	5	3	3	3	3	3
	E.M.	5	4	5	3	3	3
	S.A.	4	3	3	5	3	3
	H.W.	5	5	3	3	3	5
Hyman	W.R.	3	4	3	3	3	3
	P.L.	5	5	4	5	5	4
	E.S.	5	3	3	3	3	3
	S.	5	3	5	5	4	2
Davison	P.C.	3	1	0	1	0	0
	M.H.	2	2	2	2	0	2
	L.H.	2	2	1	2	0	0
	M.M.	2	1	0	1	2	0
Average values		4.06	3.19	3.13	3.19	2.75	2.56

*These ratings were derived according to the evaluation code which Dr. Rockwell et al used, as stated in a letter from him April 25, 1949, but which was omitted in error from the original manuscript. This code is given in detail on page 450.

The certification of allergenic extracts based on methods of standardizing that require the use of human volunteers for skin testing or passive transfer tests present certain practical difficulties. They require the co-operation of many allergists and numerous volunteers. Considering the number of allergenic extracts used by allergists it would appear impossible to enlist the large army of allergists and volunteers that would be required. In this investigation, for example, in which only six extracts were studied, letters were sent to eighteen prominent members of the American College of Allergists in an effort to enlist their co-operation in this project. Of this number fifteen replied that they would be able to help, but with varying degrees of reservation, and three indicated that they had no available time. Dr. F. W. Wittich, Secretary of the American College of Allergists, was asked to include in one of his newsletters a request for volunteers to help in this project. Replies were received from fourteen allergists. Of the group of fifteen prominent members of the American College of Allergists, samples were mailed to ten. Of these ten allergists, six responded with reports, one stated that he had lost his protocol, and three failed to reply. Of the fourteen allergists who responded to the newsletter request, extracts were mailed to one, and reports were received from him. Of the total of seven allergists who sent in reports, only one allergist responded in less than a month after we had mailed the extracts, four allergists in from one to three months, and two allergists in three months or more. This is not intended as a criticism of the allergists who offered to help in this project but serves rather as evidence that the co-operation of overworked allergists could not be expected in a certification program based upon skin testing of human beings.

SUMMARY AND CONCLUSIONS

Six house dust extracts were prepared, analyzed for total nitrogen and for phosphotungstic acid nitrogen by the methods of Rockwell, Thomas and Wittich, and tested for skin potency by the intradermal injection of persons sensitive to house dust. In confirmation of their findings and of those of others, no correlation was found between the skin potencies of the extracts and their total nitrogen or P.T.A. nitrogen content.

The six extracts were found to be approximately equal in molecular size. When they were diluted to the same phosphotungstic acid nitrogen content and tested for skin potency by the intradermal injection of persons sensitive to house dust they failed to elicit skin reactions of the same magnitude. This finding is in contrast to the findings of Rockwell et al.

Since the active substance responsible for the allergenic potency of dust extracts is not known, methods of standardization that are based on determinations of nitrogen in any form cannot be depended upon to yield reliable information concerning the allergenic potency of these extracts.

The certification of allergenic extracts on the basis of a method of standardizing that requires the use of human volunteers for skin testing is not feasible.

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ANNIVERSARY OF MEDICAL JOURNAL

The ANNALS OF ALLERGY acknowledges receiving a copy of the Spring Issue of the Hebrew Medical Journal, *HAROFE HAIVRI,* Volume I, 1950, of their 23rd anniversary year. This volume contains various subjects of interest to our readers. The Journal, edited by Moses Einhorn, M.D., is written in Hebrew with English summaries. In the current number a symposium is presented on various phases of disease and health in Israel. Among the articles of interest are "Orthopedic Problems in Israel" by I. Pulvermacher, M.D.; "Fighting Deafness in Israel" by Ahron Schwarzbart, M.D.; and "The Labor Health Service in Israel" by Moshe Rabinowitz of Tel Aviv.

STANDARDIZATION OF DUST EXTRACTS

II. In Vitro Leukocytolysis in the Assay of the Allergenicity of Dust Extracts

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THERE is general agreement among allergists that methods of standardizing allergenic extracts on a biological basis are to be preferred to any chemical method. Unfortunately, the only biological methods that are acceptable today are those which involve the skin testing of human beings known to be sensitive to the allergens to be standardized. These methods do not lend themselves readily to the testing of large numbers of allergenic extracts, as would be required in a certification program. An attempt has, therefore, been made to develop a method of standardizing allergenic extracts by the use of animals instead of human beings.

In developing the method, some reaction was sought that was common to both allergy in human beings and anaphylaxis in animals. It was hoped that if some quantitative relationship could be found to exist between such a reaction in human beings and in animals, it could possibly be used to standardize allergenic extracts.

The findings of Squire and Lee (1947) appeared to point to such a possibility. These authors reported that heparinized blood from a ragweed-sensitive patient when incubated with ragweed antigen caused lysis of the white cells approximately in proportion to the degree of sensitivity of the patient. It was reasoned, therefore, that the degree of lysis might be also proportional to the concentration of active principle in the antigen. Katz (1940) had shown that heparinized blood from sensitized rabbits, upon incubation with homologous antigen, caused a marked increase in the amount of plasma histamine, while blood from non-sensitive rabbits and plasma from sensitive rabbits failed to show such an increase; and, since Code (1937) had showed that the major portion of the blood histamine is contained in the leukocytes, it was reasoned that the histamine released in Katz's work may have been from leukocytes that had undergone lysis. If it could be proved that the leukocytolytic reactions that occur in rabbits are analogous to those that occur in human beings it was felt that rabbit cell leukocytolysis might be used as a means of standardizing allergenic extracts.

EXPERIMENTAL

Preliminary Experiment Using Blood from Rabbits Sensitized with Egg Albumin.—A preliminary experiment was run in an attempt to demonstrate this phenomenon of leukocyte destruction, using egg albumin as an-

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TABLE I. PERCENTAGES OF DECREASE IN THE NUMBER OF LEUKOCYTES NOTED AFTER INCUBATING EGG ALBUMIN SENSITIVE RABBIT'S BLOOD WITH DILUTIONS OF EGG ALBUMIN

Concentration of Egg Albumin in Blood from Sensitized Rabbit	Number of Leukocytes	Percentage of Decrease in the Leukocytes
1–10	2800	58.8
1–100	4016	40.9
1–1000	4950	27.2
1–10000	5850	14.0
saline	6800	
1–200	2050	61.6
1–400	2250	58
1–800	2800	47.6
1–1600	3550	33.6
1–3200	3900	27.1
saline	5350	
1–2000	4000	23.8
1–4000	4650	11.4
1–8000	4800	8.6
1–16000	5200	1.0
1–32000	5300	0
saline	5250	

tigen and the heparinized blood from rabbits sensitized with this antigen.

A rabbit was injected intravenously with 1 ml. of 10 per cent solution of egg albumin every third day until a total of 4 ml. had been injected. Twenty-one days after the last injection, 9 ml. of blood were drawn from the marginal ear vein into a calibrated centrifuge tube containing 1 ml. of a stock heparin solution (25 mg. of heparin in 10 ml. of 0.85 per cent saline solution). The tube was stoppered and inverted several times to mix. Forty-five hundredths milliliter amounts of heparinized blood were then added to chemically clean serological tubes containing 0.05 ml. amounts of varying dilutions of egg albumin in saline, and to one tube containing 0.05 ml. of physiological saline solution. These tubes were then stoppered with corks, gently inverted six times, and placed in a water bath at 37° C. for sixty minutes. The tubes were then removed from the water bath and again inverted gently six times. White cell counts were made from the diluted blood in each tube in the usual way. The experiment was performed three times using concentrations of 1-10, 1-100, 1-1000, and 1-10,000 of the egg albumin in the blood the first time, 1-200, 1-400, 1-800, 1-1600, and 1-3200 the second time, and 1-2000, 1-4000, 1-8000, 1-16000, and 1-32000 the third time.

As a control, blood from two normal rabbits that had not been previously injected with egg albumin was examined in the same manner as above with the exception that the 1-2000, 1-4000, 1-8000, 1-16000, and 1-32000 dilutions of egg albumin were not used. The percentage of decrease of these controls was found to be less than 5 per cent.

The results of this preliminary experiment with the blood from the sensitized rabbit are given in Table I. As may be seen from this table, the blood from the rabbit that was sensitized to the egg albumin showed leukocytolysis, upon *in vitro* incubation with egg albumin, in approximate proportion to the concentration of the antigen. The trend indicated in each of the three attempts is not a constant one; that is, there is no consistent

quantitative relationship between the concentration of egg albumin and the percentage of decrease in the number of leukocytes. The consistent failure of the blood from normal rabbits to show this marked decrease in the number of leukocytes indicates that this phenomenon is the result of a specific antigen antibody reaction.

*Experiments with Rabbits Injected with House Dust Extracts.**—Ten rabbits were injected with house dust extracts in the following manner:

Rabbit 5M was injected with house dust extract B.B. which had been preserved with 50 per cent glycerin. One milliliter of this extract was injected intravenously every third day until a total volume of 4 ml. had been given. Rabbits 6 and 8M were injected with house dust extract M.R. which had been preserved with 50 per cent glycerin. One milliliter of this extract was injected intravenously into each rabbit every third day until 4 ml. had been given.

Rabbits 9 and 10 were injected with house dust extract M.S. which had been preserved with 50 per cent glycerin. One milliliter of this extract was injected intravenously into each rabbit every third day until a total of 4 ml. had been given.

Rabbits 5R, 8R, 7, and 11 were injected intracutaneously once a week for thirteen weeks with 0.5 ml. amounts of a mixture consisting of one part of house dust extract and 1 part of staphylococcus toxin. The staphylococcus toxin was obtained from a sterile broth filtrate of a forty-eight-hour culture of *Micrococcus pyogenes* var. *aureus*, obtained from the stock culture collection of the Department of Bacteriology at the University of Kentucky. It was shown by Burky (1934) that it is possible to render rabbits sensitive to ragweed antigen by either injecting the filtrate of a *"Staphylococcus aureus"* culture that had been grown in a broth containing ragweed extract, or by injecting toxin in one site and ragweed extract in another. In our work the toxin was given with the dust extract in a mixture of equal parts of toxin and house dust extract M.R.

Rabbit 14 was injected with an emulsion of house dust extract, oil, and heat-killed *Mycobacterium tuberculosis*. This emulsion was prepared according to the method of Freund and McDermott (1942). Aquaphor and heavy paraffin oil were sterilized in the autoclave. The bacterial culture was autoclaved at 15 pounds for fifteen minutes. The growth was removed from the surface of the coagulated egg medium and the organism dried *in vacuo* and weighed. Ten milliliters of dust extract M.S. plus 10 ml. of the sterile aquaphor were blended in a sterile Waring Blendor, and 20 ml. of the sterile paraffin oil, containing 40 mg. of the dried *Mycobacterium tuberculosis* var. *hominis*, strain H. 37 R.V., were added and blended. The resulting antigen was aseptically transferred to sterile vials. Five-tenths milliliter amounts were injected intraperitoneally into each rabbit once weekly for eleven weeks.

*For a description of these extracts see the paper by Scherago, Berkowitz, and Reitman (1950).

TABLE II. RESULT OF LEUKOCYTOLYSIS EXPERIMENT WITH RABBIT "SHOCK BLOOD" AND SERIAL DILUTIONS OF BOTH HOMOLOGOUS AND HETEROLOGOUS DUST EXTRACTS

Blood from Rabbit Number	Sensitized to Dust Extract	Tested with Dust Extract	Leukocyte Count and Per Cent Decrease in Number of Leukocytes with Dust Extract Diluted						Saline Control
			1-10	1-20	1-40	1-80	1-160	1-320	
6	M.R. glyc.	M.S.	*	5900 45	5750 46.5	6550 39.1	7550 29.8	7850 27	10750
6	M.R. glyc.	M.R.	*	3150 53.3	4100 39.2	4750 29.7	4350 35.6	4800 28.9	6750
6	M.R. glyc.	M.R.	2050 46.6	3050 20.8	2900 24.7	3100 19.5	2700 30	*	3850
6	M.R. glyc.	M.R.	*	5550 32	5200 36.2	7500 8	5700 30	6800 16.5	8150
8R	M.R. S.t.	M.R.	*	4550 46.5	5700 33	6050 28.8	6600 22.3	6650 21.8	8500
8R	M.R. S.t.	M.R.	*	5700 46.3	6950 34.5	7350 30.6	9250 12.7	9250 12.7	10600
8R	M.R. S.t.	M.R.	*	6300 37.6	7350 27.2	7550 25.2	6500 35.6	7200 28.7	10100
8R	M.R. S.t.	M.R.	*	5450 46	5050 50	7850 22.3	6600 34.6	7750 23.1	10100
8M	M.R. glyc.	M.R.	6150 38	6850 31.8	9450 5.3	9450 5.3	9250 7	8950 10	9950
8R	M.R. S.t.	B.B.	5000 54.7	5450 50.7	8250 25.4	7300 33.9	12100 0	*	11050
5M	B.B. glyc.	B.B.	8350 14.7	7050 28	9000 8.1	9850 0	10600 0	*	9800
10	M.S. glyc.	M.S.	*	5800 25.6	8000 0	7350 5.8	7450 4.5	7500 3.8	7800
9	M.S. glyc.	M.S.	*	8150 41	11550 16.3	12500 9.4	10850 21.4	11750 14.9	13800
N-1	none	M.R.	5600 .89	5550 1.8	4600 18	5650 0	5350 5.3	5650 0	5650
N-2	none	M.R.	*	9900 6.6	10400 1.8	12000 0	11250 0	10200 3.8	10600
N-3	none	M.R.	*	9650 1.7	10100 0	9300 5.1	10200 0	9450 3.5	9800

Glyc. = injected with glycerine.
S.t. = injected with Staphylococcus toxin.
*Not tested with that dilution.

In from four days to one week after the last injection 10 ml. samples of blood were obtained aseptically from each rabbit by cardiac puncture. The sera were separated from the clots and transferred aseptically to sterile test tubes for later use (see Experiments 3, 4, and 5b). Enough crystalline merthiolate was added to each tube of serum to make a final concentration of 1-1000.

1. Leukocytolysis experiments using serial dilutions of house dust extracts and blood from house dust sensitive rabbits:

Rabbits 5M, 6, 8R, 9, and 10 were utilized in this experiment. Twenty-one days after the last injection of dust extract, each rabbit was bled from the marginal ear vein into a calibrated centrifuge tube containing 0.5 ml. of the stock heparin solution until 5.5 ml. of blood were obtained. Forty-five-hundredths milliliter amounts of a sample of blood were added to

tubes containing 0.05 ml. amounts of a 1-2, 1-4, 1-8, 1-16, and 1-32 dilution of one of the following dust extracts: M.R., B.B., or M.S., and to a control tube containing 0.05 ml. of physiological saline solution. Each tube was stoppered with clean corks, inverted gently six times, and placed in a water bath at 37° C. for sixty minutes. After this period of incubation the tubes were removed from the water bath and again inverted gently six times, after which samples were withdrawn from each tube into leukocyte diluting pipettes for counting. After the counts were made in the usual manner, the percentages of decrease in the blood-dust extract mixtures as compared with the blood-saline controls were determined. Samples of blood from three rabbits (N_1, N_2, and N_3) that had not been previously injected with dust extract were used as controls and tested in the same manner as the blood from sensitive rabbits.

The results of the leukocyte counts, accompanied by the percentages of decrease obtained, are shown in Table II. As may be seen from this table, no significant decrease resulted with any of the dilutions tested when blood from noninjected (control) animals (N_1, N_2, and N_3) was used. Of thirteen trials with the blood from the dust-injected rabbits the highest percentage of decrease in the number of leukocytes was obtained with the lowest dilution of dust extract in nine trials, and the least percentage of decrease in the number of leukocytes was obtained with the highest dilution of dust in seven trials. In these experiments the trend showing leukocytolysis in approximate proportion to the concentration of antigen is not of a quantitative nature. Nevertheless, the decrease that occurred corresponds roughly to the concentration of antigen.

2. Leukocytolysis experiments using 1-2 dilutions of house dust extracts and blood from rabbits sensitized to dust:

Since the degree of leukocytolysis in the animals injected with house dust extracts corresponded roughly to the concentration of dust extract with which the blood of these animals was tested, this experiment was conducted in an attempt to see if these shock bloods would reveal differences in potency among the extracts tested. Rabbits 6, 8M, and 9 were used in this experiment. Forty-five-hundredths milliliter amounts of heparinized blood from each rabbit were added to tubes containing 0.05 ml. amounts of a 1-2 dilution of the six house dust extracts and to a control tube containing 0.05 ml. of saline solution. The tubes were then inverted, incubated, and inverted again; and the leukocytes were counted as in the previous experiment, except that counts were made in both counting chambers instead of in only one, and the number of cells in the two chambers was averaged. This precaution was taken in the hope of obtaining more consistent results. In Experiment 1 it was shown that a particular dilution of extract in a given trial gave a greater percentage of decrease than a more concentrated dilution. By counting more cells it was felt that this error might be minimized.

The results of the leukocyte counts, together with the corresponding

TABLE III. RESULTS OF LEUKOCYTOLYSIS EXPERIMENT WITH WHOLE RABBIT "SHOCK BLOOD" AND DUST EXTRACTS

Blood from Rabbit	Leukocyte Count and Per Cent Decrease in Number of Leukocytes with Dust Extract						Saline Control
	M.R.	B.B.	M.S.	J.H.	M.H.	R.W.	
8M	6400 28.5	6150 31.3	7600 15.3	9450 0	6950 22.4	8200 8.4	8950
8M	6750 14	6200 21	7000 10.8	7850 0	6950 11.5	7100 9.5	7850
8M	6600 13.2	6300 17	6800 10.5	7700 0	*	*	7600
8M	7250 18.1	6900 22	6450 27	7850 11.3	6100 31	7150 19.2	8850
6	6300 31.6	6700 27.2	6900 25	9100 1.1	6450 29.9	7550 17.9	9200
6	6450 13.5	5600 24.8	5950 20.1	5750 22.8	6400 14.2	6150 17.4	7450
6	5300 31.6	5300 31.6	5850 24.6	6550 15.5	7350 5.1	6900 10.9	7750
9	5650 42	5500 43.5	6200 36.5	6750 30.8	6550 32.9	7450 23.6	9750
9	6950 31.2	5900 41.5	6500 35.6	6900 31.6	6000 40.5	7200 28.7	10100
9	6250 27.3	6850 20.4	5900 31.4	7100 17.5	6900 19.8	7650 11	8600
Average percentage of decrease	25.1	28	23.7	13.1	23	16.3	
Average deviation from the mean	±2.6	±2.2	±2.3	±3.3	±2.8	±1.6	

*Not tested with that extract.

percentages of decrease obtained in this experiment, are recorded in Table III. The percentages of decrease for each extract from ten trials were averaged and the errors of these average values were calculated. As may be seen from this table the average values of the percentages of decrease descend in magnitude in the following order: B.B. 28, M.R. 25.1, M.S. 23.7, M.H. 23, R.W. 16.3, and J.H. 13.1 per cent. If the extracts are arranged in order of potency on the basis of the skin test values* obtained with them the following sequence is obtained: B.B. 3.22, M.H. 3.16, M.R. 2.84, M.S. 2.76, J.H. 1.97, and R.W. 1.57. The skin test potencies and the average leukocytolysis values of the extracts are shown graphically in Figure 1. As may be seen from this graph, extracts B.B., M.R., M.S., and J.H. show the same relative potencies when tested by both of these methods.

3. The demonstration of specific leukocytolysis using *in vitro* sensitized rabbits' blood:

The results of Experiment 2 indicated considerable variation in the percentage of decrease evoked by a single extract from one trial to another. This error was dramatized by determining the reliability of the average values obtained as represented by the average deviation of the mean for each dust extract. The average deviation of the mean values are recorded

*See Table IV in the first paper of this series by Scherago, Berkowitz, and Reitman. Ann. Allergy, 8:437-452, 1950.

in Table III. As an example of this variation, extract M.R. has an average percentage decrease value, determined by averaging ten trials, of 25.1 per cent. The individual values in the ten trials ranged from a low of 13.2

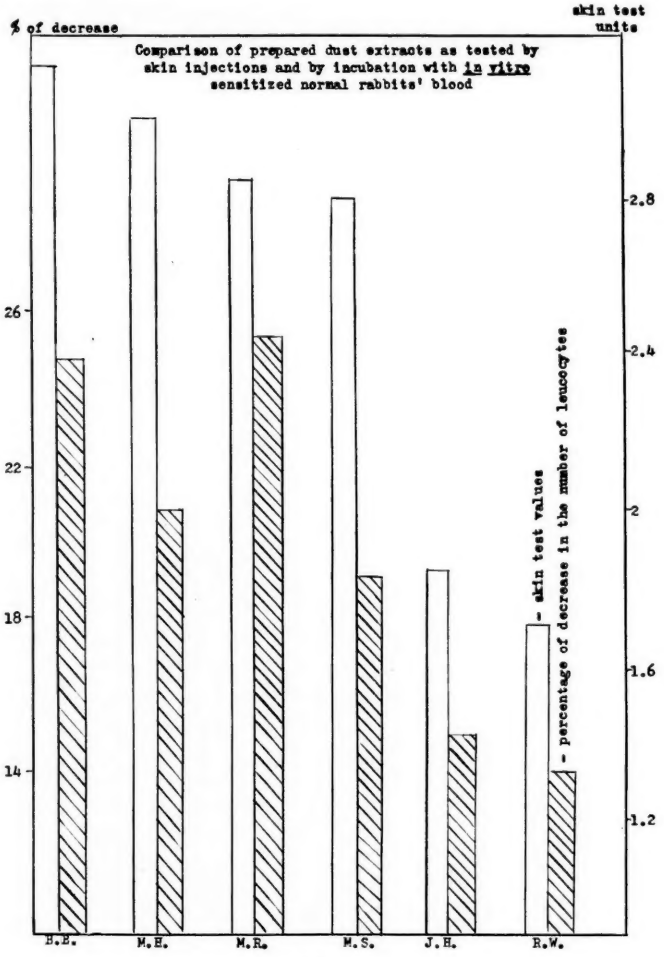

Fig. 1

per cent to a high of 42 per cent. A possible explanation for this error was thought to be the different degrees of sensitization of the particular animal for that day and the number of leukocytes present in that particular animal for that day. To remedy this partially an attempt was made to sensitize, *in vitro,* leukocytes from a normal rabbit by incubating this rabbit's whole blood with dilutions of an antiserum against house dust extract that had

TABLE IV. PERCENTAGES OF DECREASE IN THE NUMBER OF LEUKOCYTES OBTAINED USING IN VITRO SENSITIZED NORMAL RABBITS' BLOOD PREPARED WITH DIFFERENT DILUTIONS OF RABBIT HOUSE DUST ANTISERA

Anti-serum*	Dust Extract Used for Testing	Leukocyte Count and Per Cent Decrease in Number of Leukocytes in Antiserum Dilution					Saline Control
		1-10	1-20	1-40	1-80	1-160	
8R	M.R. glyc.**	4150 30.8	3950 34.2	2850 52.5	3700 38.4	5450 9.2	6000
8R	M.R. glyc.	5400 37.7	4600 46.8	4000 53.8	4800 44.5	7900 8.7	8650
8R	M.R. glyc.	5300 0	4900 0	3950 20.2	4650 6	4800 3	4950
8R	M.R. glyc.	5000 24.2	4350 34.1	4150 37.2	4450 32.6	4500 31.8	6600
8R	B.B. glyc.	5750 12.9	5500 16.7	4650 29.8	5400 18.2	6000 9.1	6600
8R	M.R. glyc.	4350 17.9	3650 30.8	3550 33	4050 23.6	4050 23.6	5300
5R	M.R. glyc.	4700 28.8	3900 41	3500 47	4850 26.5	5750 12.9	6600
8R	M.R. glyc.	5400 17.5	4850 26	5100 22.2	5050 22.9	5750 12.2	6550

*The antiserum designation is derived from the animal supplying the serum.
**Glyc. =preserved with glycerine.

been prepared in another rabbit. Blood was obtained from normal rabbits in the usual manner and heparinized. Forty-five-hundredths milliliter amounts were added to serological tubes containing 0.05 ml. of undiluted, and 1-2, 1-4, 1-8, and 1-16 dilutions of dust extract antisera from rabbits 8R, 5R, and 8M, and to a control tube containing 0.05 ml. of saline solution. These tubes were cork-stoppered, inverted six times, incubated at 37° C. for sixty minutes, and again inverted six times, after which 0.05 ml. of house dust extract diluted 1-2 were added to each tube. The tubes were again inverted, incubated, inverted again, and counted in the usual manner. The results of eight such determinations are recorded in Table IV. From this table it can be seen that the maximum amount of leukocytolysis was obtained when a final concentration of 1-40 antiserum in blood was used. Any greater concentration of antiserum resulted in a prozone phenomenon.

4. Assay of dust extracts by means of *in vitro* sensitized blood:

Since it was found possible to sensitize passively the leukocytes of blood from normal rabbits with rabbit dust extract antisera, an attempt was made to determine the potencies of the six dust extracts by incubating them with blood thus sensitized. The sensitization for these experiments was accomplished by placing 3.6 ml. of heparinized blood in a ⅝ by 5 inch test tube containing 0.4 ml. of a 1-4 dilution of rabbit dust extract antiserum. The tube was stoppered, inverted six times, and incubated at 37° C. for sixty minutes, after which it was again inverted six times. Forty-five-hundredths milliliter amounts of the sensitized blood were then transferred to serological tubes containing 0.05 ml. amounts of the six dust

TABLE V. THE PERCENTAGES OF DECREASE IN THE NUMBER OF LEUKOCYTES OBTAINED BY INCUBATING PREPARED HOUSE DUST EXTRACTS WITH IN VITRO SENSITIZED RABBITS' BLOOD

Blood of Animal Used	Sensitizing Antiserum used (dilution 1-40)	Leukocyte Count and Per Cent Decrease in Number of Leukocytes with Dust Extract						Saline Control
		M.R.	B.B.	M.S.	J.H.	M.H.	R.W.	
N-1	8R	5950 29.6	4600 45.5	6200 26.6	6650 21.4	5500 34.3	6600 21.9	8450
N-2	5R	5750 19.6	5150 28	6650 7	6500 9.1	6300 11.9	6200 13.3	7150
N-2	5R	3700 32.8	3900 29.1	3900 29.1	5100 7.3	3750 31.8	4050 26.2	5500
N-1	5R	6550 20.6	5600 31.5	6600 20	6500 21.4	6850 17	7200 12.7	8250
N-2	5R	5900 19.7	5450 25.9	6750 8.1	6700 8.8	6900 6.1	6500 11.5	7350
N-1	5R	6100 33.7	6450 30	6250 32	7200 21.8	6650 27.8	7750 15.8	9200
N-3	5R	6300 24.7	6400 22.9	6850 17.5	7700 7.1	6750 18.7	7650 8	8300
N-2	5R	6900 19.7	7250 15.7	7000 18.3	7250 15.7	7550 12.6	7850 9.1	8600
N-1	5R	3750 46.9	3750 46.9	4450 36.9	5500 21.9	4750 32.6	5650 19.8	7050
N-2	5R	6850 22.6	7450 15.8	7100 19.8	8300 6.2	7150 19.2	7400 16.4	8850
N-3	8R	4900 17.7	5400 9.2	4800 19.3	4800 19.3	5150 13.4	4900 17.7	5950
N-1	5R	6950 27.3	7000 26.7	8250 13.6	8250 13.6	8100 15.2	8700 8.9	9550
N-2	5R	6350 35	6400 34.4	6550 32.6	6850 29	6700 31.2	7650 21.5	9750
N-3	5R	5100 25.6	4700 31.4	6050 11.3	6250 8.7	5050 26.3	5800 15.5	6850
N-1	5R	6700 8.2	6850 6.1	6350 13	7250 .7	5850 19.2	7300 0	7300
N-2	5R	6750 14.6	7100 10.1	7150 9.5	6650 15.8	6950 12	7100 10.1	7900
N-3	5R	4450 23.3	4000 31	4800 17.3	4850 16.4	4600 20.7	5200 10.7	5800
N-1	8R	4600 30.3	5100 22.8	5150 22	5300 19.7	5000 24.2	5450 17.5	6600
N-2	8R	4900 31.4	5300 25.9	5950 16.8	6300 11.9	5300 25.9	6200 13.3	7150
N-3	8R	4400 30.1	5300 15.9	5450 13.5	5300 15.9	4900 22.2	5650 10.3	6300
Average percentage of decrease		25.6	25.2	19.2	14.1	21.1	14.0	
Average deviation from the mean		±1.5	±1.8	±1.4	±1.3	±1.5	±1	

extracts, and to one tube containing 0.05 ml. of saline solution. These tubes were then inverted, incubated at 37° C. for sixty minutes, and then reinverted, as was done in the preceding experiment. Leukocyte counts were then made from the contents of each tube. The entire procedure as outlined was repeated twenty times. The results of these twenty trials and the percentages of decrease in the number of leukocytes are recorded in Table V. The average percentages of decrease for each dust extract and

the errors of these average values were calculated and are also given in Table V. As may be seen from this table, the average percentages of decrease show considerable improvement in reliability as compared to the

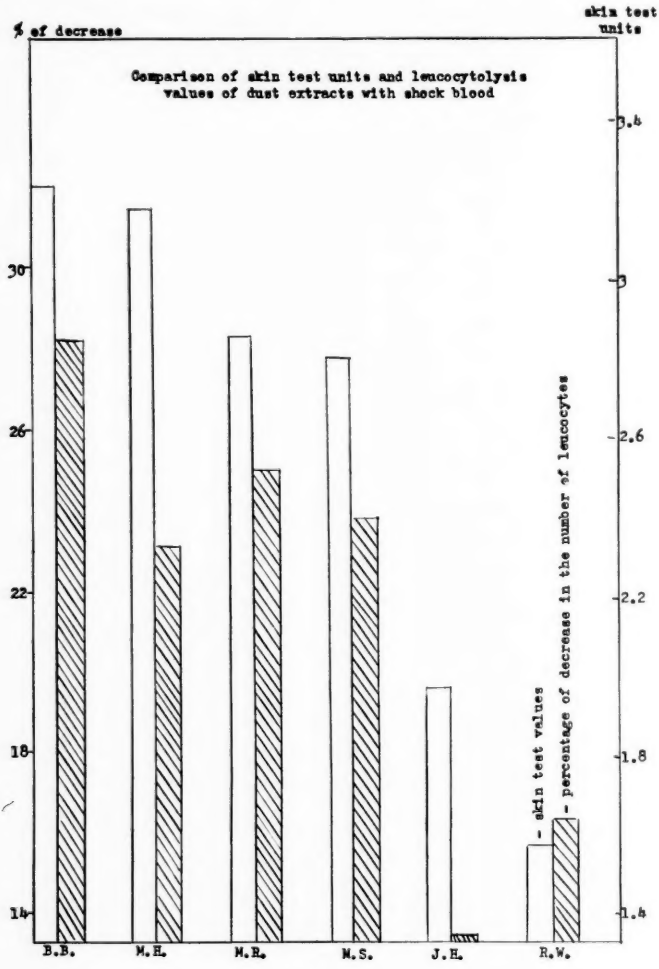

Fig. 2

results obtained using whole shock blood from anaphylactically sensitive rabbits. The maximum error recorded in this experiment is 1.8 as compared to a maximum error of 2.8 recorded in the experiment using whole shock blood. The average values obtained by using *in vitro* sensitized cells show the extracts to range in potency as follows: M.R. 25.6, B.B. 25.2, M.H. 21.1, M.S. 19.2, J.H. 14.1, and R.W. 14. These average values of

TABLE VI. PERCENTAGES OF DECREASE IN THE NUMBER OF LEUKOCYTES OBTAINED USING COMMERCIAL DUST EXTRACTS AND BLOOD FROM SENSITIZED RABBITS

Animal Used	Leukocyte Count and Per Cent Decrease in Number of Leukocytes with Dust Extract							Saline Control
	9200	CAC-1	CAC-2	CAC-3	CAC-4	19916	1A50-B	
9	6800 23.2	8950 0	7300 17.5	8000 9.6	8850 0	5750 35	8200 7.2	8850
9	7450 15.3	8000 9.6	8250 6.8	7750 12.4	8400 5.1	6000 32.2	8050 9.1	8850
9	8200 16.8	8850 10.2	9000 8.7	9050 8.1	9550 3	6250 36.6	8150 17.3	9850
9	7850 22.6	9900 2.5	9250 8.8	9250 8.8	10750 0	7050 30.5	7450 26.6	10150
9	7600 21.2	8250 14.5	7000 27.5	8350 13.5	7950 17.6	5600 42	7550 22.8	9650
9	7350 6.4	8350 0	10650 0	7300 7	8150 0	5900 25	7000 10.8	7850
9	10350 10	8800 23.2	10250 10.5	10550 8.3	9950 13.1	7200 37	9300 18.8	11450
7	6250 12	6400 9.9	6900 2.8	6450 9.1	6950 2.1	4450 37.4	6400 9.9	7100
11	7450 20.8	9050 3.7	8950 4.8	8100 13.8	9050 3.7	5750 38.8	7500 20.2	9400
14	5600 26	6350 15.9	6500 13.9	6400 15.2	6800 9.9	4450 41	5900 21.8	7550
9	7050 23.4	8850 3.8	8150 11.4	7500 18.5	8500 7.6	5850 36.4	8150 11.4	9200
7	6450 18.9	7550 5	7200 9.4	7100 10.7	7400 6.9	4700 40.9	7450 6.3	7950
9	18650 8.8	19900 2.7	20350 .4	19100 6.6	18050 11.7	14400 29.6	16650 18.5	20450
Average values	17.3	7.8	9.4	10.9	6.2	35.5	15.3	

percentage of decrease for each extract are plotted in graph form in Figure 2 together with the skin test values for each extract. As may be seen from this graph, the six extracts with the exception of extract M.R. show the same relative potencies when tested by both of these methods.

5. Assay of commercial house dust extracts by *in vitro* leukocytolysis:

An attempt was made to apply the *in vitro* leukocytolysis principle to the assay of commercial house dust extracts. For this purpose seven commercial extracts were obtained from four manufacturers as indicated below.

Samples C.A.C.-1, C.A.C.-2, C.A.C.-3, and C.A.C.-4 were obtained from Company CAC. These samples were labeled "preservative 50 per cent glycerin."

Sample 9200 was obtained from Company CCY. This sample was labeled "preserved with 0.4 per cent tricresol."

Sample 1A50-B was obtained from Company LD. This sample had no indicated preservative.

Sample 19916 was obtained from Company PC. This sample was labeled "0.5 per cent in 50 per cent glycerin."

TABLE VII. THE PERCENTAGES OF DECREASE IN THE NUMBER OF LEUKOCYTES OBTAINED USING COMMERCIAL DUST EXTRACTS AND IN VITRO SENSITIZED RABBITS' BLOOD

Blood of Rabbit Used	Sensitizing Antiserum Used (Dilution 1-40)	Leukocyte Count and Per Cent Decrease in Number of Leukocytes with Dust Extract							Saline Control
		9200	CAC-1	CAC-2	CAC-3	CAC-4	19916	1A50-B	
N-1	5R	4200 28.2	5450 6.8	6100 0	6350 0	5150 12	3650 37.6	5150 12	5850
N-2	5R	5150 18.2	7050 0	6400 0	6150 2.3	5450 13.5	5050 19.8	4850 23	6300
N-1	5R	6000 21	8200 0	8050 0	7950 0	7450 2	5950 21.8	6350 16.5	7600
N-2	5R	6850 20.8	9950 0	7950 8.1	8500 1.7	7600 12.1	4500 48	6850 20.8	8650
N-1	5R	5450 23.8	6200 13.3	6700 6.4	6450 9.8	6900 3.5	4800 32.8	6900 3.5	7150
N-1	5R	5850 12.7	6850 0	6800 0	6600 1.5	6350 5.2	4850 27.6	6400 4.5	6700
Average values		20.8	3.3	2.4	2.5	8.1	31.1	13.3	

(a) Assay with whole blood from sensitized rabbits: The seven extracts were tested by adding 0.45 ml. amounts of heparinized shock blood to serological tubes containing 0.05 ml. amounts of the samples. These tubes were incubated and counts were made as in the preceding experiments. The results of thirteen trials are recorded in Table VI. As may be seen from this table, the seven extracts, on the basis of percentage decrease of leukocytes, range in potency as follows: 19916 35.5, 9200 18., 1A50-B 15.3, CAC-3 10.9, CAC-2 9.4, CAC-1 7.8, CAC-4 6.2. For control purposes, the seven extracts were tested with the blood of normal rabbits which had not been previously injected with house dust. In no case was there any leukocyte destruction greater than 5 per cent in these controls.

(b) Assay with *in vitro* sensitized blood from normal rabbits: Heparinized blood from normal rabbits was sensitized *in vitro* with dust extract rabbit antiserum in the same manner as in Experiment 4. The sensitized blood was incubated with the commercial dust extracts and counts were made as was done in Experiment *a*. The results of the leukocyte counts accompanied by the percentages of decrease observed for the seven extracts as tested in six different trials, are recorded in Table VII.

As may be seen from this table, the seven extracts on the basis of percentage decrease of leukocytes range in potency as follows: 19916 31.1, 9200 20.4, 1A50-B 13, CAC-4 8.1, CAC-1 3.3, CAC-3 2.5, CAC-2 2.3

Thus, by both methods of assay extracts 19916, 9200, 1A50-B ranged in potency in that order. The CAC extracts by both methods were the least potent. However, the four extracts from this company did not react in the same order by both methods. By the first method they ranged in the following order CAC-3, CAC-2, CAC-1, CAC-4; and by the second, CAC-4, CAC-1, CAC-3, and CAC-2.

DISCUSSION

The *in vitro* bio-assay procedure for determining the allergenic potencies of house dust extracts that was developed in this investigation was based on the following considerations. Histamine is a mediating substance in both anaphylaxis in animals and allergy in human beings. Katz (1940) had demonstrated that whole blood from sensitive rabbits released enough histamine upon *in vitro* shock to play a significant role in anaphylaxis, and Squire and Lee (1947) had showed that leukocytolysis occurred when heparinized blood from ragweed sensitive patients was incubated with ragweed pollen extracts. It was hoped, therefore, that a quantitative relationship might be found to exist between the degree of leukocytolysis of cells from dust-sensitized rabbits and the concentration of the active fraction in the dust extracts being tested, comparable to the quantitative relationship that exists between human skin sensitivity and the concentration of this fraction.

Later in the investigation, advantage was taken of the report of the success of Dragstedt, Arellano, Lawton, and Youmans (1940) in passively sensitizing, *in vitro*, the blood of normal rabbits, to modify the procedure by substituting for the blood of sensitized rabbits blood from normal rabbits that was passively sensitized, *in vitro*, with the serum from immunized rabbits.

Before applying the leukocytolysis bio-assay procedure to the standardization of dust extracts a preliminary experiment was conducted with egg albumin, an antigen of proven potency, in order to test the method and to become proficient in the technique. The results of this experiment revealed that although the percentages of decrease in the number of leukocytes were not absolutely in proportion to the concentrations of the antigen, in general, the greatest amount of leukocytolysis occurred when the higher concentrations of antigen were used and the least amount of leukocytolysis when the lower concentrations were used. These results appeared to be sufficiently encouraging to warrant the testing of the method with house dust extracts.

The first experiment with house dust extract, which was essentially a repetition of the egg albumin experiment, yielded approximately the same results as were obtained with the egg albumin. Here again, in general, the lower concentrations of dust extract caused the lower decrease in the number of leukocytes and vice versa. That the leukocytolysis observed was not due to some leukotoxic substance in the dust extract was shown by the relative absence of leukocytolysis in the control experiments in which blood from normal rabbits was substituted for the blood from the sensitized ones.

Despite the lack of absolute correlation between the percentage of decrease in the number of leukocytes and the concentration of egg albumin or dust extract, it was thought worth while to determine the relative potencies of the six dust extracts by the method of leukocytolysis and to com-

pare them with the relative potencies of these extracts as determined by skin tests on dust-sensitive patients. When the six extracts were arranged in order of potency on the basis of leukocytolysis (Tables III and V, and Figs. 1 and 2), this arrangement was approximately, though not absolutely, comparable to the arrangement of the extracts on the basis of their skin reactivity. For example, extracts B.B., M.R., M.S., and J.H. showed the same relative potencies when they were tested by their ability to produce leukocytolysis in shock rabbits' blood as they did when they were tested by skin reactivity. This correlation between the extracts was even closer when *in vitro* passively sensitized rabbits' blood was substituted for blood from actively sensitized rabbits. Extracts B.B., M.H., M.S., J.H., and R.W. showed the same relative potencies by both methods.

In the course of the experiments with the blood from sensitized rabbits considerable variation was noted in the percentages of decrease with a particular dust extract from one experiment to another of the same type. In an effort to minimize this error, blood from normal rabbits that was passively sensitized, *in vitro*, with rabbit house dust antisera was substituted for the blood from sensitized rabbits. It was reasoned that the different rabbits used as the source of shock blood, regardless of how they were sensitized, each had a different degree of sensitivity, and this variation in sensitivity might have been responsible for the large degree of variation in our early experiments. In an effort to obtain leukocytes with a more constant degree of sensitization, blood from normal rabbits was sensitized, *in vitro*, with a constant amount of antiserum. The results obtained by this modification showed some improvement as evidenced by the decrease in the error obtained. The maximum error with blood from sensitive rabbits was plus or minus 2.8 while with the passively sensitized blood it was no more than plus or minus 1.8.

Although the *in vitro* bio-assay procedure developed in this investigation did not yield results which would point to the applicability of this method in its present stage of development to the standardization of dust extracts, it is felt that the results are sufficiently encouraging to warrant further investigation and, perhaps, modification of the method. It is felt that the principles upon which this method is based appear sounder than those of the chemical methods.

Among the modifications to be tried is the use of standard suspensions of leukocytes that have been separated from the other blood cells and have been sensitized, *in vitro*, with a standard amount of a standard antiserum. The means of determining the efficacy of this procedure might also be modified by using a serum neutralization procedure for this purpose instead of skin sensitivity tests. This would eliminate the discrepancies arising from the varying degrees of sensitivity in sensitive persons. With these modifications it is possible that the results obtained will be consistent enough to have quantitative significance.

SUMMARY AND CONCLUSIONS

A method of standardizing dust extracts was developed based on the fact that leukocytes from the blood of sensitized animals, or leukocytes from the blood of normal rabbits that had been passively sensitized *in vitro* with serum from sensitized rabbits, are lysed when they are incubated *in vitro* with the homologous antigen. When this method was applied to the standardization of six dust extracts, it was found that on the basis of the per cent of decrease in the number of leukocytes the relative potencies of the extracts paralleled closely their relative potencies on the basis of their skin reactivities. Closer agreement was obtained when passively sensitized cells were used instead of cells from sensitized rabbits.

The leukocytolysis method, in its present state of development, cannot be used to standardize dust extracts. However, the results obtained are encouraging enough to warrant further study of this method with certain suggested modifications.

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ERUPTION FOLLOWING HOME PERMANENT

The consensus of investigators who have conducted toxicologic studies on these products [home permanent wave sets] is that when reactions occur they usually take the form of a contact dermatitis. Goldman, and others (Permanent Wave Process, *J. A. M. A.* 137:354 [May 22] 1948) stated: "Cold waving solutions are used extensively, and the frequency of reactions is reported to be less than 0.1 per cent. The reactions may be characterized either as varying degrees of primary irritation or, more rarely, as instances of eczematous hypersensitivity." The use of these products in the presence of an existing dermatitis is inadvisable. Representative manufacturers include a caution statement to this effect in their directions for use.—From Queries and Minor Notes, J.A.M.A., July 22, 1950.

THE IMMUNOLOGICAL PROPERTIES OF ALCOHOL
A Survey of the Literature

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RECENT increased interest in the problem of chronic alcoholism and renewed investigation of the concept that there is a biological basis for the development of this disease have led to an extensive review of the literature for evidence that ethyl alcohol may have allergenic or antigenic properties. Many of these reports have never been indexed in this country; indeed, few have been cited in the English language. Thus it appears timely to review the reports which have appeared since the first serotherapeutic method for alcoholism was proposed in 1896, to evaluate so far as possible their immunological significance and to point out some of the investigations necessary to any confirmation of the antigenic or allergenic properties of ethyl alcohol.

To clarify this discussion, the following definitions have been adopted:

1. *Chronic alcoholism:* a disease characterized by continued incapacitation of the subject for normal life because of steady or periodic ingestion of alcohol. Chronic alcoholism may be primary or may be secondary to other physical or mental disorders, but in this discussion primary chronic alcoholism is the usual concern.
2. *Tolerance:* the ability of the individual to resist the *characteristic* response to alcohol. In this review, resistance to the physiological effects of alcohol is the predominant concern.
3. *Intolerance:* The existence of a very low resistance to the *characteristic* response to alcohol.*
4. *Allergy or hypersensitivity:* a specific but abnormal response of the individual to alcohol, wherein it acts as an antigen and the response is due to the toxic effects of the *in vivo* formation of a specific antigen-reagin complex.
5. *Susceptibility or abnormal reaction:* an abnormal response of the individual to alcohol, including responses that are less definite manifestations of allergy (e.g., excessive motor activity, nervousness, et cetera).
6. *Habituation:* a state of being accustomed to the regular ingestion of alcohol, irrespective of whether tolerance is increased or decreased, or whether the response is normal or altered.
7. *Need:* dependence on the ingestion of alcohol. Physiological need implies a demand for alcohol created by a specific adaptation of the body which makes the substance "valuable." Physiological need is not a corollary of tolerance (which is known to exist during periods of abstinence without involving the occurrence of disturbing reactions) but should be considered to be the expression of an abnormal reaction to alcohol. Psychological need may be considered to be the expression of an abnormal desire for the effects of alcohol irrespective of tolerance, susceptibility or physiological need.

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*The term "intolerance" has been used by many to denote both lack of tolerance and susceptibility. Since such dual usage leads to confusion, the reviewer has interpreted the term in accordance with the context of its use: when a direct quotation is employed, the proper connotation is indicated.

8. *Craving:* a term used to denote both physiological and psychological need. Its use will be avoided, but where necessary the proper connotation will be indicated.

ALLERGIC REACTIONS TO ETHYL ALCOHOL

The first to suggest that abnormal reactions to and need for alcohol resulted from the development of a reagin ("antibodies," to quote the author) was Ashworth (1929-32[6,7,8,9]). He considered periodic sprees as "explosions" resulting from an accumulation of reagin; and withdrawal symptoms, experienced in breaking alcohol addiction, as the result of dissemination of the irritating reagin throughout the body. This explanation of the occurrence of sprees was most original, but no experimental evidence was offered to support it. It is difficult to see how any accumulation of presumably unbound reagin could be responsible for the phenomenon, especially since it is implied that the accumulations occur during periods of abstinence when no antigen is available to stimulate reagin production. Similarly, Ashworth's explanation of withdrawal symptoms would be more adequate if the symptoms were the result of an antigen-reagin complex which persists for some time after its formation.

Both Silkworth (1937[60]) and Lee (1938[32]) stated that alcoholism is an allergic reaction. Silkworth treated it with an "appropriate colloidal preparation such as . . . orthocolloidal iodine complex or orthocolloidal gold" to "revitalize the cells," a very nonspecific treatment. Cowles (1941[20]) likened the edema of nerve cells after administration of alcohol to the nasal turgescence found in pollen allergy and treated chronic alcoholism by repeated withdrawal of spinal fluid to lessen the edema. At first glance Seliger (1939[58]) and Shadel (1944[59]) may seem to hold similar views. Actually, they have drawn an analogy between the "household" term "allergic reaction" and alcoholism only as a vivid means of emphasizing the importance of abstinence to patients, but not with intent to imply that allergy to alcohol exists.

In 1947 Meerloo[43] reported a death from acute intoxication which resembled anaphylaxis and summarized the evidence which has led many to speculate that the sudden susceptibility to alcohol which develops in chronic alcoholics may be similar to the development of a protein allergy.

> It is still theoretical whether we are allowed to speak of a real alcoholic allergy, as we speak of some protein allergies. Indeed, we have to prove that there first is an initial sensitizing dosage and that the second reaction is quantitatively and qualitatively different from the initial one. However, in medical literature the word is used in a much wider sense. Even when we do not know how some drugs act as sensitizers and what kind of allergens are produced, the clinical facts as such exist. For example, the Herxheimer reaction after neosalvarsan intoxication is explained in this way. Barbiturates and chinin have such a sudden allergic reaction. Nirvanol in children may give the same reaction. Some students think that the drug changes the proteins of the body and that an allergic reaction develops as a reaction towards this deformed protein. Clinically it is known that several narcotic drugs develop their intolerant [abnormal—Reviewer] reactions only in combination with certain foods, meat, fish, etc. Especially with drugs applied to the skin, many allergic

reactions are observed (resorcinum, iodoform). With some drugs this allergic factor should be related to the CH_3 group or the NH_2 group. With other drugs it is known that the allergy develops as a photogene allergy, i.e., only in combination with intense ultraviolet rays. Epstein could prove this form of photogene allergy for sulfanilamide. It is generally known that the exogenous irritant can be changed in the body into an allergen.

What do some clinical facts tell us about alcohol? Here, too, exists a form of sudden intolerance [susceptibility—Reviewer] with a complete different clinical reaction. The patients react with collapse after a relatively small quantity of alcohol. There is tremor, vomiting, dizziness, nystagmus, ataxia, tremendous hiccup. The patients have a pale face with a typical mask of roughly spread vasodilations, but there is no change in consciousness. Days afterwards they cannot bear the smell of alcohol and once they experience such a reaction, for long periods they become dizzy soon after relatively small quantities of alcohol.

Where do we meet such reactions?

We see them in chronic alcoholics.

Lemere and his associates[33] likewise stated in 1943 that "susceptibility to alcohol is often constitutional and akin to an allergy or an idiosyncrasy to a drug."

There have been two detailed descriptions of the allergic activity of ethyl alcohol. In 1938 Perlman[49] reported its occurrence in a patient who, during skin tests by the scratch method, reacted to alcoholic extracts of allergens, but did not react to nonalcoholic extracts of the same allergens when nonalcoholic antiseptics were used for skin disinfection. The patient, a middle-aged nun with a history of symptoms strongly suggestive of allergic rhinitis and asthma, stated that there were no signs of hypersensitivity to the ingestion of small quantities of alcohol during religious rites. It was considered improper to attempt a provocative test by asking her to imbibe any alcohol and, since she moved to another city, further studies could not be carried out.[50]

In 1945 Haxthausen[27] reported a case of eczema elicited by epidermal contact with ethyl alcohol. The eczema disappeared on cessation of contact and could be subsequently elicited at will either by epidermal application of alcohol or by ingestion of alcoholic beverages. Patch tests with varying concentrations of alcohol showed the strongest reactions in the range of 10 to 20 per cent; intracutaneous injection of 1 or 2 per cent ethyl alcohol in saline gave no reaction. Four months later this patient was examined by Lomholt[37] and reactions were elicited only by absolute, by 91 per cent and by 60 per cent ethyl alcohol. In the interval between examinations the patient, a physician, had substituted nonalcoholic antiseptics for disinfecting her hands, and it is apparent that her sensitivity decreased with the lessened exposure. It would be interesting to know how she would react to patch tests if exposure to alcohol was increased again.

Additional impetus to the idea that an allergic reaction may be involved in the response to alcohol comes from the alcohol susceptibility skin test. Bonazzi reported in 1934[14] that application of alcohol-soaked patches to the slightly scarified skin of subjects produced intense local reactions lasting

three to six hours in non-drinkers but very little reaction in habitual drinkers. In 1939 Nagle[16] reported employing intracutaneous injections of ethyl alcohol to study this reaction in both nonalcoholic and alcoholic subjects. According to his report, subjects showing the strongest reaction to this test exhibited the greatest psychological impairment after the ingestion of alcohol; conversely, those showing the least reaction exhibited the least impairment, i.e., possessed the greatest tolerance to alcohol. Nagle suggested that the test could be used as a guide in the regulation of alcohol intake by patients undergoing treatment for alcoholism. After making similar observations in 1941, Kelley and Barrera[29] concluded that reactions to the alcohol susceptibility skin test vary inversely with tolerance to alcohol when the results of the tests are correlated with subjective and objective observation of individuals having varying concentrations of alcohol in the blood. After commenting on Silkworth's interpretation of the nature of alcoholism, they state:

> Such an hypothesis of alcoholism, as an allergic condition resulting from a physiological sensitivity, would help explain individual differences in alcohol susceptibility on the basis of varying inherent sensitivity and could account to some degree, for tolerance changes and increasing susceptibility with prolonged indulgence. Furthermore, it would serve as a basis for better understanding of the mechanism of the alcoholic susceptibility test.

Although the consensus is that a strong reaction to the alcohol susceptibility skin test signifies low tolerance and a weak reaction, high tolerance, it has not been shown that there is any relationship between the degree of the reaction and the occurrence of abnormal responses to alcohol. Since Nagle stated that the pharmacological response is less (i.e., tolerance is greater) in those habituated to alcohol and that the response was the same in all subjects tested, he failed to differentiate between normal but regular drinkers and abnormal drinkers, or between abnormal drinkers who have suffered no loss of tolerance and those whose tolerance has decreased. To sustain the postulate of Kelley and Barrera that alcoholism is an allergic condition and that the alcohol susceptibility skin test supports the postulate, it should be shown that reactions to the test differentiate between normal and abnormal drinkers with equal tolerance to alcohol. Until this has been shown, or until the complete mechanism of the alcohol susceptibility skin test has been demonstrated, it does not seem proper to consider that the test provides more than an indication of variations in tolerance to alcohol.

Summary.—The two reports on systemic response to alcohol and the theoretical papers reviewed here may not warrant conclusions, but they afford some basis for the idea that an allergic reaction to alcohol may enter into the problem of the individual response to alcohol.

ANTIGENIC ACTIVITY OF ETHYL ALCOHOL

The preparation and use of antialcoholic sera, with their implication of an antigenic activity of ethyl alcohol, antedate the idea of allergic reactions

to alcohol by many years. It was quite natural that, shortly after methods of serotherapy were developed, the possibility of their application to the scourge of alcoholism should have been considered. The basic hypothesis for this method of treating alcoholism was stated best by Sapelier and Dromard:[56] "If it is true that an antitoxic substance is developed in the blood of an animal submitted progressively to alcoholic toxins, might this substance not be utilized in another animal suffering from the same intoxication to aid it in combatting the intoxication?"† Although this hypothesis seemed logical at that time it needs verification and amplification for the following reasons: (1) It fails to explain why continued ingestion of alcohol does not provoke formation of sufficient antibody to make the habitual drinker immune to its effects, or why, after a period of apparent partial "immunity" to its effects, the "immunity" may suddenly disappear. (2) It fails to account for physiological need for alcohol, since known antibodies or reagins have not been shown to cause physiological dependence on an antigen. (3) It does not take into account the possibility that alcohol may be allergenic rather than antigenic. A survey of the various methods of preparing antialcoholic sera and of their use is essential for evaluating the antigenicity of alcohol.

PREPARATION OF ANTIALCOHOLIC SERA

In 1896 Toulouse[64,65] reported the preparation of the first antialcoholic serum. He gave dogs about 40 gm. of ethyl alcohol per day for six days; fasted them the seventh day and on the eighth day bled them. No mention was made of any further treatment of the serum, or of the performance of toxicity tests before it was administered to a patient.

The blood and serum of horses which had ingested alcohol were commonly used for the treatment of alcoholism. F. M. Evelyn of San Francisco (as reported in 1899 by Caze[18] and in 1900 by Regnier[52]*) developed Equinine, blood impregnated disks of filter paper for application to the skin of alcoholics. Horses were given two to three pints of whisky per day for about three months and when microscopic examination of the blood showed the red cells to be "thick, viscous and syrupy," the horses were bled. Filter paper was then dipped in the blood and dried at high temperature.

The first report on antialcoholic horse serum was made in 1899 by Broca-Soucellier, Sapelier and Thébault,** and in 1903 its preparation was described in detail by Sapelier and Dromard.[56] Horses were given moderate, increasing doses of ethyl alcohol, twice a day, until a maximum dose of about 500 gm. was reached on the tenth day. Sapelier and Dromard specified that no animal be used which did not accept alcohol willingly, and that the animals show no ill effects or even excitation from the doses.

†Reviewer's translation.

*No reference can be found to Evelyn's original article. These citations both refer to an account by Caze, M.: Vaccination contre l'ivrognerie. Hyg. usuelle, (Jan. 14) 1899, which is not available in the United States.

**This report was published only by title in the Bull. Acad. Méd., Paris, Dec. 26, 1899, and all information comes from the book by Sapelier and Dromard.[56]

These stipulations arose from their belief that the animals should approximate the human state of latent alcoholism, or "alcoholomania," for which the serum was to be employed. By the tenth day, examination of the blood usually showed that the red cells were losing the regularity of their shape, ceasing rouleaux formation, and appearing to agglutinate or be "coupled with each other;" that the white cell count was greatly increased; and that "fatty granules" (refractile granules of the eosinophiles?) were present, or were greatly increased. When this blood picture appeared, blood was drawn aseptically and the serum recovered. The serum was sealed in ampules and heated three times, at two-day intervals, for one hour at 56° C. Sapelier's criterion for the absence of toxicity in the serum was the lack of any reaction by guinea pigs to administration of 30 c.c. of serum. Whether further inoculation and bleeding of the horses was done was not stated. Sapelier and Dromard objected strongly to increasing the titer by *in vitro* treatment of the serum (Broca-Soucellier†) on the grounds that the activity of the serum must be due to antibody titer attained during exposure to alcohol if the serum was to be considered an antiserum.

In the period following the work of Sapelier and his associates, methods of preparing antialcoholic sera for therapeutic use were less well described. Blasco in 1905,[13] Delfino in 1913[21] and Berillon in 1919[11] referred to preparation of antialcoholic horse serum at the Instituto Ferran de Sagrene (Barcelona) but gave no details. Delfino did cite Ferran's claim that if the serum was kept from light and heat, it might retain 10 per cent of its activity for a year and a half. Delfino also mentioned an auxiliary product for oral reinforcement of serotherapy, called "antiethylene hemoglobin with stroma of red blood cells," which was prepared by Ferran from the blood of horses which had been accustomed to alcoholic drinks. This information suggests that Ferran used alcoholic beverages rather than ethyl alcohol in preparing his sera. Hernandez (1912[28]) mentioned that Acosta, at the laboratory of the *Crónica médico-quirurgica* of Havana, prepared antialcoholic serum as a public service, but no report of Acosta's method has been found.

Bertarelli (1932[12]) stated briefly that antialcoholic horse serum was prepared at the Laboratorio Paulista de Biologia (Brazil) by giving horses oral doses of aguardiente until large amounts could be tolerated. After several months the horses were bled and their serum used. He suggested that the serum should be made polyvalent by giving the horses various types of liquors, or that sera should be prepared employing different liquors so that sera homologous to the preferred liquor of the patient would be available, since the alcohol might not be responsible for the effects of the serum—a thought echoed by Carratala.[17] Apparently antialcoholic sera of this type are available in Latin America today, for *"Sôro antialcoólico L.P.B."* was listed among the biological products available from the Lab-

†No description of this method of increasing the titer could be found, but it was implied to be a chemical method.

oratorio Paulista de Biologia in 1938,[61] and in 1944 an advertisement for it was interposed in the text of an article on its use by Santiago.[55]

Autoserum[1,24,38] prepared from the blood of the patient has also been used in Latin America. The patient was instructed to continue ingesting his normal daily amount of alcoholic beverages. Blood was drawn in the morning before the first drink, and the recovered serum was injected subcutaneously or intramuscularly later the same day. This serum was, therefore, similar to that secured from horses given alcoholic beverages but, according to its proponents, it obviated the danger of reactions caused by injection of heterologous serum. Continuing with the idea of avoiding the use of heterologous serum, but with the view that it would be advisable for the patient to cease drinking during the treatment, Pareja C. (1947[47]) prepared alcoholized human serum. He withdrew 10 c.c. of blood from the patient and removed the serum from the clot the next day. To 5 c.c. of serum he added 1 c.c. of the alcoholic beverage preferred by the patient and, after shaking and centrifuging the mixture, the supernate was injected intramuscularly. If the serum was not to be used immediately after its preparation, a preservative was added. Later Pareja C.[48] reported that, since it did not seem necessary to practice strict autoserotherapy and because the patients objected to frequent venipuncture, the procedure was modified to making a single withdrawal of 400 c.c. of blood, which provided enough serum for about sixteen injections. He also varied the alcoholic products used for alcoholization of the serum and concluded that aguardiente de caña, of about 36 proof in 30 per cent concentration, produced the best results.

In addition to the various serum preparations for therapeutic use, several investigators have produced serum for experimental purposes only. After waiting for the further reports promised by Toulouse,[65] Maramaldi[40] decided in 1898 to investigate the two problems Toulouse had proposed, namely, whether the alcohol should be given in more gradual doses over a longer period of time, and whether normal serum would be as effective as antialcoholic serum. Maramaldi began by giving 1 c.c. of 90 per cent ethyl alcohol per kg. of body weight, suitably diluted to prevent local irritation, by stomach tube to a dog; he increased the dose every ten or eleven days. After four months, when the dog was receiving daily doses of about 6 c.c. of alcohol per kg., it was bled. Bleeding was repeated when the animal was receiving about 7.5 c.c. per kg., and it was bled out a few days later after several doses of 8 c.c. per kg. The blood was collected aseptically and kept cold for twenty-four hours before the serum was removed, but no other treatment of the serum was reported.

In 1914 Manoiloff[39] reported preparing antialcoholic serum in rabbits for use in the production of passive sensitization to anaphylaxis. Commencing with 0.2 c.c. of 60 per cent alcohol, and increasing the dose by 0.1 c.c. each time, he gave rabbits ethyl alcohol intravenously every other day until a daily dose of 1.5 c.c. was reached. This series of injections was then re-

peated, using next 80 per cent and finally 95 per cent alcohol.* At the end of the third series of injections the rabbits were bled and the serum was inactivated before use.

The most recent reports on production of antialcoholic serum are those of Loiseleur (1946[34]) and Loiseleur and Levy (1947[35]), who are studying the antigen-antibody reactions of organic molecules of low molecular weight. They gave injections of 95 per cent ethyl alcohol to rabbits, beginning with 70 mg. of absolute alcohol per day and increasing to a maximum of 7,200 mg. at the end of thirty to forty days. Believing that the difficulty in producing antibodies to molecules of low molecular weight might arise from the rapid excretion of these very small molecules, they used large volumes of diluent, frequent injections, and the intramuscular route to maintain as constant a presence of the antigen in the body as was feasible. In the case of alcohol, each dose was diluted in physiological saline to approximately 30 c.c. and given in two to four equal parts equally spaced throughout twenty-four-hour periods, a procedure designed to make the animals approximate the state of continual alcoholic impregnation encountered in chronic alcoholics. The animals were bled the day after the last injection because it was found that the activity of the serum diminished rapidly once the injections ceased. There was no further processing of the serum, and no mention was made of the effect of storage on its activity. More recently, Loiseleur and Sauvage[36] have reported that more satisfactory sera may be produced by twice daily injections of 10 c.c. of a mixture of equal parts absolute ethyl alcohol and physiological saline over a period of ten to twenty days and that the titer of the serum will vary with the length of time the injections are continued. They also reported that the optimal time for bleeding the animals was three to four days after the last injection, a time long enough to allow complete disappearance of free alcohol from the body but not so long that the antibody titer would drop appreciably.

Summary.—The methods of producing antialcoholic sera for therapeutic use appear to have been more haphazard than those for laboratory experimentation, but there is a possibility that methods have been kept secret for commercial reasons. The later reports show a trend toward longer immunization periods and this trend seems practical because such periods usually yield greater amounts of antibody.

There is little reason to criticize the heating of the antialcoholic sera for purposes of inactivation of the complement or for pasteurization, but if Evelyn's "high temperature" was greater than 56° C., it is doubtful that any active antibody remained after heating. If the antialcoholic antibody should be stable at 52° to 56° C., heating the serum was desirable since this would decrease its toxicity.

*It is difficult to believe that these concentrations were actually used, since they would be too injurious to the veins to allow so many injections. Ahlquist[5] noted that even 20 per cent ethyl alcohol in 5 per cent glucose solution was not satisfactory for repeated intravenous injections.

TABLE I. PROTOCOL OF MARAMALDI'S EXPERIMENTS*

Weight of Animal g.	Amount 90% Ethyl Alcohol Given	Amount 90% Ethyl Alcohol per kg.	Serum				Observations
			Bleeding	Dose c.c.	Route	Time after Alcohol	
4500	51 c.c.	12 c.c.	May 25	8	Intraperitoneal	3 min.	Cured after 19 hrs.
			May 25	8	Intraperitoneal	3 hrs.	
			May 25	10	Intraperitoneal	9 hrs.	
5000	60 c.c.	12 c.c.	May 25	10	Intraperitoneal	4 min.	Cured after 30 hrs.
			May 25	7	Intraperitoneal	3 hrs.	
			May 25	4	Subcutaneous	17½ hrs.	
6100	79 c.c.	13 c.c.	July 6	30†	Intraperitoneal		Died after 15 hrs.
6400	84 c.c.	13 c.c.	July 6	15	Subcutaneous	4 min.	Cured after 30 hrs.
			July 6	10	Subcutaneous	3 hrs.	
			July 6	5	Subcutaneous	9 hrs.	
			July 6	10	Intravenous	20½ hrs.	
7200	93 c.c.	13 c.c.	July 6	10	Intraperitoneal	4 min.	Cured after 22 hrs.
			July 6	10	Intravenous	40 min.	
			July 6	10	Intravenous	5 hrs.	
7000	98 c.c.	14 c.c.	July 6	35†	Intraperitoneal and intravenous		Died after 14 hrs.
7500	105 c.c.	14 c.c.	July 6	15	Intraperitoneal	5 min.	Cured after 26 hrs.
			July 6	15	Subcutaneous	3½ hrs.	
			July 6	10	Intraperitoneal	17½ hrs.	
7300	110 c.c.	15 c.c.	July 18	15	Intraperitoneal	5 min.	Cured after 24 hrs.
			July 18	10	Intraperitoneal	5½ hrs.	
			July 18	10	Intraperitoneal	17½ hrs.	
6800	100 c.c.	15 c.c.	July 18	15	Intraperitoneal	4 min.	Cured after 24 hrs.
			July 18	10	Intraperitoneal	4½ hrs.	
5500	88 c.c.	16 c.c.	July 18	40†	Intraperitoneal		Died after 15 hrs.
6800	108 c.c.	16 c.c.	July 18	50†	Intravenous		Died after 18 hrs.

*Modified from Table of Maramaldi. (43).
†Only total dose reported.

The severest criticism of most antialcoholic sera produced for therapeutic use is that alcoholic beverages rather than pure ethyl alcohol were employed in their production. This difficulty, intimated by Bertarelli's[12] desire for a polyvalent serum, was better interpreted by Bahamonde Q. (1944[10]), who pointed out that some persons have true allergic reactions to certain types of liquors but not to others—reactions which are stimulated by substances other than ethyl alcohol. Thus the use of sera prepared by giving animals liquors does not differentiate between reactions caused by the presence of antibodies to ethyl alcohol and reactions caused by the presence of antibodies to other substances. Hence the only sera that may be considered as possibly having properties derived from the presence of antibodies to ethyl alcohol are those prepared by Toulouse, by Maramaldi, by Sapelier and his associates, by Manoiloff, and by Loiseleur and his fellow workers. It is unfortunate that Pareja C., who has supplied better case reports and is also the newest author in this field, did not use ethyl alcohol to alcoholize his human serum preparations.

EXPERIMENTAL WORK WITH ANTIALCOHOLIC SERUM

In 1898 Maramaldi[40] reported the first and best controlled animal experiments with antialcoholic serum. First, he determined the minimal lethal dose of 90 per cent ethyl alcohol for dogs to be 12 c.c. per kg. of body weight when given by stomach tube, and that in all cases death occurred in less than eight hours. He next gave 1 to 1.25 M.L.D. of ethyl alcohol to dogs and three to five minutes later injected the first dose of serum. Additional doses of serum were given at varying intervals (Table I). Of nine dogs thus treated, seven survived. Dogs which received more than 1.25

M.L.D. of alcohol died after fifteen to eighteen hours despite the fact that they were given much larger doses of serum. In all cases death occurred much later in treated animals than in control animals. Finally to demonstrate that the antialcoholic serum was responsible for the survival of the treated animals, 1 M.L.D. of ethyl alcohol was administered to two dogs and then injections of normal serum were made, one being given 40 c.c. and the other 50 c.c. Both dogs developed intoxication of the same intensity and died, as had the control animals, in a little more than three hours. This led Maramaldi to conclude that the antialcoholic serum contained an antitoxin capable of neutralizing the toxic action of 1.25 M.L.D. of ethyl alcohol. He observed that intravenous injection of the serum relieved disturbances of heart rate and respiration more rapidly than intraperitoneal injections, but the duration of the intoxication was the same with either route of administration. Because it was unnecessary to increase the dose of serum appreciably upon increase of the dose of alcohol, Maramaldi assumed that the serum obtained from the later bleedings of the serum-producing dog (used to treat the animals that received the larger doses of alcohol) contained more antitoxin than the earlier lots; he suggested that methods of producing a more potent antitoxin should be investigated.

Three isolated experiments on the use of antialcoholic serum in animals that had been habituated to consuming food or water containing alcohol were cited by Dromard.[22] One animal failed to alter its consumption of food or water and two reacted favorably. Sapelier and Dromard[56] mentioned that studies were also made in guinea pigs to observe the effect of the treatment on pregnancy and the resulting progeny, but they did not report any results. The experiments seem to indicate that there was some substance present in the antialcoholic serum which, upon exposure of the treated animals to alcohol, caused a new type of reaction to it—that of avoidance of substances containing alcohol—but the nature of the reaction was not suggested.

In 1914 Manoiloff[39] reported that he and Zboromirsky** apparently had produced anaphylaxis in rabbits and guinea pigs by administering intravenous doses of alcohol forty-eight hours after passive sensitization with serum from chronic alcoholics. This finding led him to prepare an antialcoholic rabbit serum which he tested in five rabbits, giving 12 to 15 c.c. of the antiserum intravenously, followed forty-seven to forty-eight hours later by intravenous injection of 0.5 to 0.6 c.c. of 95 per cent ethyl alcohol. Manoiloff stated that all the animals showed typical anaphylactic shock, and his description of postmortem findings in the one animal which died tallies with those usually associated with anaphylaxis in rabbits (Boyd).[15] Similar tests in guinea pigs (protocols not reported) gave similar results. He concluded that "serum from alcoholic animals confers passive anaphylaxis."† As noted earlier, there is some doubt about the actual concentration of ethyl alcohol injected by Manoiloff. If he used 95 per cent

**Zboromirsky and Manoiloff, or Manoiloff and Zboromirsky: Chronischer Alkoholismus und Anaphylaxie, Obozreniye Psikhiatrii, Nr. 30, 1912. Unobtainable in the United States.

ethyl alcohol for these intravenous injections, it may be questioned whether the rabbit died from right heart failure of anaphylactic origin or from thrombi elicited by the alcohol. This reviewer, however, has injected 95 per cent ethyl alcohol into rabbits intravenously and into guinea pigs intracardially without observing any evidence of thrombus formation during the period when anaphylaxis might be expected to occur. Thus, Manoiloff's conclusions do not seem to be prejudiced by this possibility.

Loiseleur employed the very sensitive method of microviscosimetry, developed by Lecomte du Noüy[30] and used for demonstrating diphtheric toxin-antitoxin reactions,[31] to detect reactions between antialcoholic rabbit serum and ethyl alcohol. He found (1946[41]) as much as 30 per cent increase in the relative viscosity of mixtures of antialcoholic serum and ethyl alcohol over that observed in normal serum-ethyl alcohol mixtures. He also found that if, after the serum of a rabbit showed such an increase in relative viscosity, the animal was given massive doses of alcohol, a decrease in the relative viscosity occurred when tests were done on serum drawn during and just after the administration of the doses, followed by an increase to a new peak. This negative phase in the relative viscosity curve, Loiseleur believed, corresponded to a temporary *in vivo* neutralization of the active principle by the excessive dose of antigen. In 1947 Loiseleur and Levy[35] reported that they had found the zone of equivalence for the maximum relative viscosity of antialcoholic serum-ethyl alcohol mixtures to be at a concentration of 0.1 mg. of absolute alcohol per cubic centimeter of serum, and that fractionation of the serum revealed that the active principle was associated with the pseudoglobulin fraction. They also found that there was considerable specificity in the reaction, for when serum, from animals injected with ethyl alcohol, was tested with methyl alcohol, the peak in the curve of relative viscosity measurements was less than one-half that with ethyl alcohol and the zone of equivalence fell at about 0.05 mg. per cubic centimeter of serum. It is interesting that in a similar study done with morphine there was also an increase in the relative viscosity of antimorphine serum-morphine mixtures as the administration of morphine proceeded; however, when massive doses of morphine were given, the relative viscosity continued to increase without any evidence of temporary *in vivo* neutralization by excess antigen. In a later paper, Loiseleur and Sauvage (1948[36]) reported that when gamma globulin, prepared from antialcoholic serum, was tested, opacity occurred in mixtures representing the zone of equivalence, and that the specificity of the reaction extended to propyl as well as to methyl alcohol.*

Loiseleur and Levy[35] speculated that physiological need (*besoin*) for both of these drugs may arise from the presence of the "antibodies" (*anticorps*) which they reported, and that therapy for such addictions may someday be achieved through the production of "anti-antibodies" (*contre-anticorps*) which would allay such need. This speculation was repeated by

†Reviewer's translation.

Loiseleur and Sauvage, who referred also to the work of Bruel and Lecoq with intravenous administration of alcohol for the relief of symptoms associated with chronic alcoholism. Since they do not explain how unbound "antibody" can cause need for the homologous antigen, and since it is more probable that an antigen-antibody or antigen-reagin complex may exist in alcoholics (at least while they are drinking), this speculation is hard to justify. A more conservative postulate would be that some abnormal reactions to these drugs arise from the formation of a reagin which, in combination with the antigen, elicits an allergic reaction (in this case the reagin shows *in vitro* reactions); or since it was not shown that the response of the rabbits to alcohol was abnormal, it may be that their serum contained an antibody and that a different mechanism is involved in abnormal physiological reactions to alcohol. Until sera from non-drinkers and from normal and abnormal drinkers are studied under the same conditions, no conclusions can be drawn about the role of the active principle described by Loiseleur in alcoholism.

Summary.—The seemingly clear-cut findings of Maramaldi, Manioloff and Loiseleur, suggesting that antialcoholic serum has some peculiar properties, necessitates confirmation of the findings and determination whether the properties are of protective (Maramaldi) or allergic (Manoiloff) character, or whether the conditions of the preparation of the serum determine its character. Such confirmation might explain the nature of tolerance to alcohol, or the altered response to alcohol, observed in chronic alcoholism, which at times resembles the development of hypersensitivity reactions. Rosenfeld (1914[54]), who studied tolerance to methyl and ethyl alcohol, did not find any increase in resistance to lethal doses of methyl alcohol after animals had been habituated to ethyl alcohol, or vice versa. This phenomenon is in accord with Loiseleur's claim of specificity for his antialcoholic serum.

THERAPEUTIC USE OF ANTIALCOHOLIC SERUM

In 1896 Toulouse[64,65] administered antialcoholic dog serum to a patient suffering from acute alcoholism with delirium tremens and found that the patient recovered more easily than was usual in such cases. The accounts of Evelyn's[18,52] use of Equinine are rather incomplete. Apparently a disk was moistened with sterile water and applied to the skin of the patient after scarification in the manner employed for smallpox vaccination. When the disk lost its color, due to absorption of the serum, a fresh disk was applied to the same area. The patient was said to lose his taste for alcohol after seven or eight applications.

From 1900 to 1903 antialcoholic serotherapy was developed by a group working with Sapelier in France. In 1900 Thébault[62] reported thirty-three

*In a personal communication, Loiseleur stated that if the mixtures of antialcoholic serum and ethyl alcohol used for the viscosimetric measurements, are incubated at 40° to 50° C. overnight, precipitation will occur, and that usually the maximum amount of precipitation will occur in the mixture representing the zone of equivalence.

successes and eight improvements in fifty-seven trials.** Dromard (1902[22]) reported success in all of thirty patients considered suitable for treatment and failure in all of ten deemed unsuitable. This French group differed from Toulouse in that they were interested in curing addiction to alcohol rather than relieving the acute symptoms of alcoholic intoxication.[56] They found the best field for this to be among latent alcoholics, or (in their term) "alcoholomaniacs," who suffered from physiological need (*accoutumance et besoin*) but did not yet show clinical evidence of the toxic effects of alcohol. In such patients, if the treatment was completed, antialcoholic serotherapy produced such a degree of repulsion and distaste for alcoholic beverages that even the sight or odor of liquor stimulated pallor, sweating, faintness, nausea and vomiting.

To account for the existence of what we call tolerance, Sapelier and Dromard postulated that alcohol possessed two active parts: one corresponding to Ehrlich's haptophore group and the other corresponding to his toxaphore group. Upon entrance of alcohol into a cell, the haptophore group was believed to cause alteration of the cell's metabolism to include production of receptors or antibodies for the protection of the cell from the toxophore group. When hyperproduction occurred, the excess receptors were released into the blood stream with resultant immunity, or tolerance, to alcohol. They thought that when the cells became accustomed to producing the antibody their metabolism was so altered that they became dependent on alcohol for nourishment, and that this nutritional requirement was the basis of physiological need. Administration of antialcoholic serum to a patient was presumed to cause the cells possessing this abnormal requirement to revert to their normal requirements, with concomitant loss of tolerance and physiological need and with re-establishment of the repulsion and distaste for alcohol which they considered the normal (nondrinker) reaction to alcohol. This explanation is weak for two reasons. First, tolerance to alcohol is considered to occur only in abnormal drinkers and is linked with physiological need. Second, in the alcoholic individual alcohol is presumed to induce the formation of an antibody responsible for immunity to the effects of alcohol, while administration of a serum prepared by giving animals alcohol results in cessation of antibody production and loss of immunity.

Of course, if more alcohol had been ingested than could be neutralized by the receptors produced by the cells, it elicited organic lesions. When this had occurred, Sapelier and Dromard felt that serotherapy was futile, because it could not be expected to restore cells which had been destroyed by the toxophore group. The duration of the repulsion varied, lasting for at least a year in some cases; but if the patient was unco-operative and forced himself to drink, the repulsion soon disappeared and relapse, accompanied by the return of physiological need for alcohol, occurred. They believed

**An identical set of figures was attributed to a report by Broca-Soucellier, Sapelier and Thébault by the editor of Le Concours Médical,[19] and was repeated by Thébault in 1901.[63] These reports apparently represent the same data.

that the occurrence of a relapse after antialcoholic serotherapy was no more valid criticism of the serum than a second attack of an infectious disease, following serotherapy for relief of the primary attack, would be of the value of the latter serum.

Sapelier and Dromard noted that serotherapy was less effective in wine drinkers than in drinkers of hard liquor; they attributed this to the fact that wine apparently more often incited digestive disorders than did other types of liquor. They did not recommend serotherapy in cases of dipsomania, other psychoses, or alcoholism associated with organic disease whether of alcoholic or nonalcoholic origin (tuberculosis, syphilis, et cetera), because alcohol or other agents had already caused irreparable damage. It is interesting to note that none of the thirty-four patients with such complications treated by Thébault[62] or Dromard[22] responded to serotherapy even though they received much more than the usual amount of serum. Sapelier and Dromard[56] particularly emphasized that suggestion had no part in the development of the repulsion, for suggestible patients uniformly exhibited little or no improvement while suitable patients who did not know that the treatment was directed toward their alcoholism developed repulsion satisfactorily. It should be noted that these authors treated chronic alcoholism on a purely physiological basis and found that their treatment was unsatisfactory when alcoholism was associated with psychotic states (dipsomania was considered to be a psychosis), i.e., the alcoholism probably was a secondary disease.

The book by Sapelier and Dromard[56] on the selection of patients and the mechanism of the reaction seems to have been responsible for the spread of the method to Spain and Latin America.[2,12,21,28] Table II summarizes the available case reports recorded since 1896.†

Use of antialcoholic horse serum has predominated, but several other preparations which might well carry a similar active principle have been employed. Hernandez,[28] while using reinjection of ascitic fluid obtained by paracentesis to treat a patient for hypertrophy of the liver with hydropoietic ascites, obtained not only an improvement of the ascites but the development of a repulsion to alcohol which resulted in the patient's abstinence until the date of the report several months later. It is interesting that Hernandez based his treatment of the ascites on the method of Galup,[26] who worked in Paris soon after Sapelier, but did not record the observation of any change in the drinking habits of patients after this treatment for ascites associated with chronic alcoholism.

Autohemotherapy[24,38,41,68] and autoserotherapy[1] seem to have yielded results comparable to those obtained with heterologous serum, although Wolffenbüttel,[68] Martimor and Maillefer[41] and Acevedo Castillo[1] reinforced the action of autohemotherapy or autoserotherapy by the use of ipecac or some other vomitant. These reports seem to contradict the find-

†More case histories probably have been published in Latin America, especially in the *Arquivos de Biologia*, but they have not been cited in American indices and the journals are not available for search. It is believed, however, that those cited may be considered typical.

TABLE II. RESULTS OF SEROTHERAPY IN CHRONIC ALCOHOLISM

Author	Date	Type of Serum	No. of Cases	Results Reported	Comment
Thebault[62]	1900	Horse (prepared with ethyl alcohol)	57	33 successful 8 improved (4 did not finish treatment, 4 showed contraindications) 16 failures (4 did not finish treatment, 13 showed contraindications)	Period of observation not stated
Dromard[22]	1902	Same	30	30 successful (all latent alcoholics)	Max. period obs. 12 mo., median 4 mo. 11 relapsed at varying intervals. Some able to drink moderately at time of last report.
			10	10 unsuccessful (all showed contraindications to treatment)	
Acosta[2 3 4]	1904 1905 1907	Horse (preparation not known)	90	70 cured and confirmed 13 extent of cure not confirmed 7 excluded (uncooperative, did not finish treatment)	Period of observation not stated, 1 year in some cases.
Blasco[13]	1905	Horse	8	2 cured, 8 mo. after inj. 1 cured 2 mo. after inj., relapsed and retreated less successfully 1 cured, relapsed at 8 mo. but drank more moderately 4 failures (1 relapsed at 8 mo., 2 abandoned treatment, 1 received serum orally)	
Hernandez[28]	1912	Ascitic fluid	1	1 cured (about 9 mo. after treatment)	
Escomel[24]	1928	Autohemotherapy	1	1 cured	Period of observation not stated
López Lomba[36]	1932	Same	24	23 successful	Period of observation not stated
Wolffenbüttel[68]	1935	Same, plus tartar emetic, ipecac, hypnosis	24	22 successful 1 abstinent for 8 years 1 abstinent for 4 months	
Martimcr & Maillefer[41]	1936	Autohemotherapy	11	5 successful	Period of observation not stated
		Same plus ipecac	45	40 successful	Period of observation nct stated
Martinez[42]	1937	Soro anti-alcoclico L. P. B.	1	1 cured—observed 2 months	
Quintero[51]	1937	Same	2	2 cured—observed 1 month	
Freire[25]	1937	Same	2	2 cured—observed 5 months	
Ribeiro[53]	1937	Same	1	1 cured—observed 2 years	
Schulhof[57]	1938	Same	1	1 cured	Period of observation not stated
Elizando[23]	1938	Same	1	1 cured	Period of observation not stated
Acevedo Castillo[1]	1943	Autoserotherapy	16	7 cured (considered method a conditioned reflex treatment) 4 improved 4 failures 1 under treatment	Period of observation not stated for each patient, range 1-14 months
Santiago[55]	1944	Soro anti-alcoolico L. P. B.	2	1 cured (abstinent) 1 improved (drinks occasionally but can work)	Period of observation not stated
Miro[45]	1944	Same	1	1 cured	Period of observation not stated
Pareja C.[47]	1947	Alcoholized human serum	16	9 cured—most cases observed 3 months 3 improved at end of treatment (2 for about 8 months) 4 failures	
Pareja C.[48]	1947	Same	111	61 definitely abstemious 21 improved 6 failures 23 abandoned treatment	Period of observation not stated

ings of Sapelier and his associates that chronic alcoholics with clinical evidence of damage from alcohol, dipsomaniacs, and the like are not benefited by serotherapy. In fact, none of the more detailed case histories indicate treatment of the mild form of addiction—latent alcoholism—and many definitely mention the presence of dipsomania or hepatic dysfunction in patients successfully treated.

The procedure in most of these methods was to permit the patient to continue his normal drinking pattern, allowing cessation of drinking to occur naturally with the development of repulsion or distaste for alcohol. Sapelier and Dromard[56] stated that it was important to allow the patient to continue his exposure to temptation, but did not definitely say that continued ingestion of alcohol was necessary or had any part in the effectiveness of the serum. In many cases patients voluntarily ceased drinking after their first injection, but were treated for some time longer; and often those who required the largest amount of serum to stop their drinking relapsed the soonest. A possible clue to the part played by alcohol in serotherapy is found in the reports of Pareja C.[47,48] who did not believe that it was right for the patient to continue drinking during the treatment. Instead, he used intramuscular injections of alcoholized patient's serum to produce the repulsion. This seems to indicate that the active element in this type of treatment may be either (1) an antigen-reagin complex which stimulates formation of the "anti-antibody" postulated by Loiseleur and Levy,[35] or (2) a protein-alcohol complex which reacts with a reagin to produce a more severe response than that produced by the formation of a similar *in vivo* protein-alcohol complex, possibly because the response is not masked by the narcotic effects of alcohol.

Summary.—The reports of the therapists who employed ipecac or apomorphine in conjunction with serotherapy must be disregarded, since these drugs alone cause nausea and vomiting and since their use makes such methods of treatment similar to the conditioned-reflex method of Voegtlin.[66] Toulouse's report of the effect of serum in acute alcoholism, however, suggests that it has protective properties and the rest of the reports suggest that serum from animals or persons who have undergone prolonged exposure to ethyl alcohol, or to substances containing it, develops a substance which, either alone or in the presence of ethyl alcohol, is capable of producing repulsion to alcohol in those addicted to its use. To date the exact mechanism of these reactions has not been investigated.

DISCUSSION

Considering the great interest that has been consistently displayed in the problem of alcoholism, it is remarkable that the experimental studies with antialcoholic serum have not been repeated under conditions which would test the validity of the claims that an alcoholic antibody or reagin may develop in the serum of animals or persons exposed to ethyl alcohol. Moreover, although the alcohol susceptibility skin test has been presumed to involve an immunological concept, no study of its mechanism or relationship to other reports of immunological reactions to alcohol has been made. The following discussion is an attempt to clarify thinking on the mechanisms which may be involved in the diverse results reported, so that the direction of further study may be more readily seen.

Correlation of Perlman's[49,50] and Haxthausen's[27] reports of cutaneous hypersensitivity to ethyl alcohol with Manoiloff's[39] report of anaphylaxis

to alcohol following passive sensitization with antialcoholic serum suggests that a substance having the properties of a reagin may exist. The phenomenon of repulsion and gastric revolt reported to be produced by antialcoholic serum in subjects habituated to alcohol would seem to indicate that the serum may contain an antialcoholic reagin, and that its administration may increase the reagin titer sufficiently to elicit a hypersensitivity reaction of such severity that the subject is restrained from drinking. On this basis, tolerance might exist when a subject does not produce a reagin to alcohol and susceptibility when he does produce one. Thus, susceptibility, whether acquired or inherited, would be an abnormal state similar to allergy and, like allergy, most easily controlled by avoidance of the causative agent.

The foregoing, however, does not account for intolerance to alcohol or for the increase of tolerance which usually occurs after experience in drinking (even when drinking is irregular). This situation would be better met by an explanation arising from the reports of Toulouse[63,64] and Maramaldi[40] that the active principle in antialcoholic serum acts as a specifically neutralizing antibody. Should this be the case, the explanation of tolerance would be reversed, for the presence of the antibody should provide protection or tolerance and its absence should cause intolerance. The alcohol susceptibility skin test does not, at present, influence either this or the previously postulated explanation of the response, for it does not differentiate between tolerance and susceptibility to alcohol. It appears to be more like the Schick test than like a skin test for allergy, but does not indicate whether "immunity" to alcohol involves a positive adaptation such as the formation of an antibody or just a negative adaptation of the body to alcohol (disregard for its presence).

By recognizing that reagin formation does not preclude antibody formation,[44] a more probable explanation appears. This is that both antibody and reagin production may occur in response to alcohol, with normal antibody formation representing the normal reaction—development of tolerance upon exposure to alcohol; lack of antibody formation (whether due to lack of exposure or failure to respond normally) representing intolerance; and abnormal antibody or reagin formation accounting for allergic reactions and possibly for some of the abnormal reactions of chronic alcoholics to alcohol. Whether alcohol alone may be antigenic or whether it may become antigenic only after combining with a protein to form a hapten has not yet been determined. The *in vitro* reactions obtained by Loiseleur[35] suggest but do not prove that it is a complete antigen. A "hapten theory" would make explanation of the existence of both antibody and reagin formation simpler, particularly if it could be shown that the form of the protein involved varies or that two or more proteins are involved.

Thus far this discussion has emphasized the relationship of a theoretical antigenic activity of alcohol to tolerance to alcohol; and of a theoretical reagin which, in combination with alcohol, might account for clear-cut

manifestations of allergy to alcohol. It is also necessary to consider the role of such a reagin in physiological need for alcohol.* As pointed out before, it is difficult to see how an antigen-reagin complex could cause a demand for the presence of more alcohol to alleviate its toxic effects, particularly the large quantities of alcohol which are demanded. The only possible explanation which can connect the existence of an antigen-reagin complex with physiological need is that the toxic effects of the complex are dulled by the normal sedative effect of alcohol. While it may be objected that, under such conditions, the sedative effect of alcohol would be sought for psychological rather than physiological reasons, it must be pointed out that Bruel and Lecoq[16] and others** have repeatedly said that intravenous injection of alcohol causes dramatic relief of delirium tremens. Oral administration of alcohol does not give this result and no other sedative acts in such a specific manner. Similarly Bruel and Lecoq found that intravenous administration of alcohol prevents the occurrence of withdrawal symptoms on abrupt cessation of drinking but that no permanent desensitization to oral ingestion of alcohol occurred. As a whole these findings tend to discourage the idea that any allergic reaction is involved in abnormal responses to alcohol by chronic alcoholics, but it may be that the unusual route of administration precluded formation of any toxic antigen-reagin complex.

In conclusion it may be said that in chronic alcoholism we have a situation wherein an apparently permanent sensitization to alcohol occurs which, at present, is relieved only by absolute and permanent cessation of contact with alcohol, including involuntary contact through such media as cough medicines and elixirs. The reports herein reviewed suggest that in some cases the sensitization may be similar to allergy and seem to warrant investigation of the following questions:

1. Does alcohol, either alone or in combination with some protein, function as an antigen or allergen?

2. If so, what are the properties of any antibody or reagin so formed? (1) What is the explanation of the sensitizing action of antialcoholic serum reported to have occurred both in animals and in chronic alcoholics? (2) What is the explanation of the therapeutic action of antialcoholic serum reported to have occurred in acute alcoholism?

3. Is the alcohol susceptibility skin test a measure of tolerance or of susceptibility, and what is its mechanism?

4. What is the mechanism of the beneficial results of intravenous administration of ethyl alcohol in delirium tremens?

*There is much discussion as to whether physiological need for alcohol exists, particularly because no satisfactory explanation for its existence has been offered. Williams, Berry and Beerstecher[67] have reported, within the last few months, that in rats and mice they have found distinctive genetic differences in response to alcohol consumption and that in certain strains of animals and certain individual animals it is relatively difficult to reduce alcohol consumption. They stated that clear evidence had been obtained that the creation of an appetite for alcohol in these animals is due to different deficiencies and that the basis of alcoholism might possibly be nutritional.

**Personal communications.

If and when any or all of these questions are answered, we shall have a basis for confirming or denying the validity of the reports of the various immunological properties of alcohol, as well as considerably more insight into the physiological portion of the problem of alcoholism.

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NORISODRINE SULPHATE (25 PER CENT) DUST INHALATION IN SEVERE ASTHMA

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SINCE the advent of epinephrine and ephedrine as adjuvants in the therapy of asthma, the search has proceeded for more effective agents productive of fewer side-effects. When, in the last decade, reports on related compounds appeared in the European literature[1,2,5,8,11,14] and later in the American literature, much interest was stirred among allergists. Encouraging reports concerning one such drug, Isopropylarterenol Sulphate, commonly known as Norisodrine Sulphate have appeared in this country since 1947.[3,4,6,7,10,12] A most interesting feature of these reports was the efficacy of this drug in the severe asthmatics who were often refractory to other symptomatic medication.

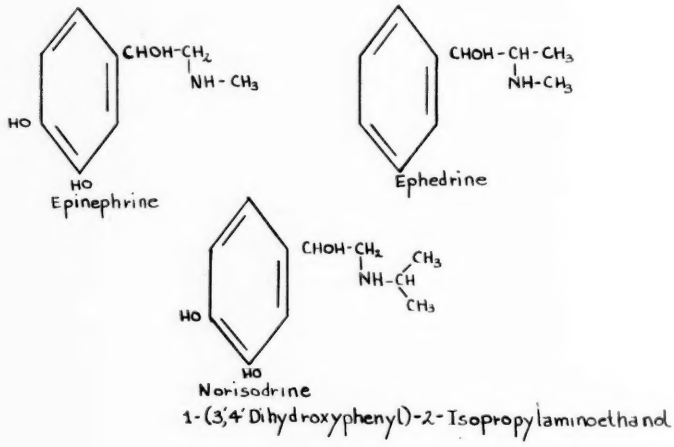

Fig. 1

The purpose of this paper is to present the results of symptomatic treatment of a dozen severe asthmatics with Norisodrine. Although this is a small number of cases from which to draw true conclusions, it is felt it will add a fragment to the accumulating data concerning the drug.

Like epinephrine, Norisodrine is a dihydroxyphenyl-ethanol amine. It differs from the former only in its alkyl group, being the isopropyl homologue of epinephrine. The structural formulas of epinephrine, ephedrine and Norisodrine are reproduced in Figure 1 for comparison.

By laboratory experiment, Norisodrine has been shown to be far less

Norisodrine Sulphate, 25 per cent Sifter Cartridges and Aerohalors were kindly supplied by the Abbott Laboratories.

toxic than epinephrine and to have a much greater broncho-dilating effect.[9,13]

Norisodrine has been used orally, subcutaneously, by hand nebulizer and dust inhalation. It was felt that for the ambulatory severe asthmatic the choice method of administration was dust inhalation (25 per cent). It is less cumbersome than hand nebulization or self injection; its effect is more rapid than the tablet; the small plastic inhaler is easily carried on the person, is unbreakable and with a little adeptness can be used in public unnoticed.

The patients selected for trial therapy were those whose treatment had been unsatisfactory in the past, whose symptoms had persisted for a long period of time and/or whose response to the more common anti-asthmatic drugs was minimal. For the most part, these cases belonged to that group so well known in every allergist's office, refractory to treatment. In addition, four mild cases of asthma were included for comparison of result. These were adequately attack-controlled by the ordinary medicaments such as epinephrine, ephedrine, aminophylline, iodides, antihistaminics alone or in combination.

Table I is an analysis of the patients and the results of treatment.

As is seen from this table, the patients ranged in age from twenty-nine to seventy. Extrinsic, intrinsic and combined types of asthma are represented. The duration of asthma was from two to thirty years, and the attack-frequency and severity were great enough to interfere seriously with the patient's day-to-day functioning. Almost all of these patients did not respond well to the more common anti-asthmatic drugs.

Of the twelve patients with severe asthma, nine experienced prolonged freedom from symptoms within a few minutes of two or three inhalations, without side effects. This was considered an excellent result. One showed a tendency to need more Norisodrine as time went on and experienced an occasional moment of palpitation, but prolonged rapid relief was obtained. This was considered a very good result. Another patient required five inhalations and the effect was not noted for ten minutes. But once relief set in, it was of considerable duration. This too was considered a good result. Another patient who responded very well to nebulized epinephrine needed two series of three inhalations of Norisodrine for relief. The effect was noticeable only after a ten-minute interval, and with it, he experienced some nausea. This patient also showed a tendency to need a greater number of inhalations as time progressed. This was considered only a fair result.

In several instances, the patient was able to discard routine daily epinephrine injections and rely on Norisodrine. This was of great benefit psychologically and from the point of view of work capacity. Dust inhalation was a matter of a few seconds and could be done in an inconspicuous manner. This obviated the former necessity of leaving the job for an injection, waiting for relief and the disappearance of side effects.

TABLE I.

			Type of Asthma				Attack			Response to Other Drugs			Response to Norisodrine (25%)							
No.	Sex	Age	Extrinsic	Intrinsic	Combined	Yrs. of Asthma	Frequency	Av. Duration	Complication	Epinephrine	Ephedrine	Aminophyline	No. Inhalations	Elapse Time	Duration of Relief	Side Effects	Period of Treatment	Inc. Tolerance	Results	Comment
1	F	40			X	4	3/Wk.	24 to 48 Hrs.	Menopause & Emotional Instability	Good and Marked Palpitation	Neg.	Neg.	3	2′	Aborts Attack	None	3 Mos.	None	Excell.	Attacks began to come on with flushes. Allergic to oral hormone also. Norisodrine prevented attacks. She takes hormones no difficulty.
2	M	44			X	3	Daily	6 to 8 Hrs.	—	Fast	Neg.	I. V. Fair	4	5′	4 Hrs.	None	3 Mos.	None	Excell.	Discarded adrenalin and syringe; works no time loss on Norisodrine.
3	F	38	X			20	2/Wk.	3 to 4 Days	Anxiety Neurosis	Fast	Neg.	I. V. Fair	3	3′	6 Hrs.	None	3 Mos.	None	Excell.	Some degree Asthma daily for 20 years. Has been free since Norisodrine.
4	M	53		X		15	1/Wk.	2 Days	—	S. C. Marked Tachy. Inh. Neg.	Neg.	I. V. Fair	3	2′	8-10 Hrs.	Occas. Palpitation	2 Mos.	Recently Inc. to 4 Inhal.	Very Good	
5	M	59			X	11	1/10 Days	12 Hrs.	G. B. Disease	Inhal. Good	Neg.	Neg.		10′	3 Hrs.	Nausea	2 Mos.	Inc. Inhal.	Fair	Only advantage here Norisodrine Inhalations simpler than Epineph Nebulization.
6	M	46		X		7	On Exertion	10′ to 3 Hrs.	Bronchiectasis	Marked tachycardia	Neg.	Neg.	5	10′	3 Hrs.	Neg.	2½ Mos.	None	Good	Relief of attack — copious expectoration.
7	M	51		X		5	82 Wks. more in Pol. Sea'n	2 Days	G. I. Allergy	S. C. Good — Side Effects	Neg.	I. V. Good	2 2	2′ 2′	Aborts Attack	Neg.	2 Mos.	None	Excell.	G. I. Allergy relieved since Norisodrine.
8	F	29	X			15	8 Week	2 to 3 Days	—	S. C. Good	Neg.	I. V. Good	2	3′	10 to 12 Hrs.	Neg.	2 Mos.	None	Excell.	
9	F	30	X			5	8 Week	1 Day	—	S. C. Good	Fair	I. V. Good	2	2′	Aborts Attack	Neg.	3 Mos.	None	Excell.	
10	M	36	X			4	Daily	10 to 14 Hrs.	—	S. C. Good	Neg.	I. V. Good	2	3′	Aborts Attack	Neg.	3 Mos.	None	Excell.	
11	M	45	X			2	8 Month	7 to 10 Days	Hypothyroid	S. C. Good	Neg.	I V. Good	4	5′	10 to 12 Hrs.	Neg.	2 Mos.	None	Excell.	
12	F	70		X		30	Daily		—	Fast	Neg.	Neg.	4	5-7′	4 to 6 Hrs.	Mild Palpitation	2 Mos.	None	Excell.	
MILD ASTHMA (Control)																				
1	F	22	X			2	8 2 to 3 Weeks	4 to 5 Hrs.	—	Excell.	Good	Good	—	—	No Relief	—	2 Mos.	—	Unsatisfactory	
2	F	31	X			1	Weekly	6 to 8 Hrs.	—	Excell.	Excell.	Excell.	—	—	No Relief	—	2 Mos.	—	Unsatisfactory	
3	M	39			X	3	Weekly	24 Hrs.	—	Excell.	Excell.	Excell.	—	—	No Relief	—	2 Mos.	—	Unsatisfactory	
4						4	2/Wk.	6 to 7 Hrs.	—	Excell.	Excell.	Excell.	3	2′	Aborts Attack	Neg.	2 Mos.	None	Excell.	

Where the patients ordinarily had recourse to intravenous aminophylline, Norisodrine inhalation obviated the need for a relatively complex procedure requiring the physician's care and gave the patient a much greater sense of security.

In Patient 1, who was suffering menopausal symptoms as well as asthma, Norisodrine aborted the "flush"-attached attack and seemed to prevent attacks that arose after the ingestion of hormone.

In Patient 6, whose asthma was complicated by bronchiectasis, use of Norisodrine not only relieved the paroxysm but also resulted in a copious flow of secretion.

It is interesting to note that in Patient 7, whose asthma was complicated by bouts of abdominal pain, eructation and nausea, Norisodrine relieved not only the asthmatic attack but also the gastrointestinal symptoms. These latter also had occurred independent of the asthmatic paroxysms but had disappeared almost entirely during the period of Norisodrine usage.

It should be stressed that during the period of Norisodrine use, none of these patients was given any other medication. Specific therapy alone was continued.

In the group of four mild to moderate asthmatic patients, all of whom responded well to epinephrine, ephedrine, antihistaminics and/or aminophylline, Norisodrine was ineffective in three, gave an excellent result in one.

Dosage of Norisodrine dust is an individual matter and is determined with each patient specifically. During the course of symptoms, the patient is instructed to take two or three shallow inhalations. Careful watch is kept for time of onset of relief and completeness of relief. If necessary an adjustment is made in the number of inhalations. Once this test dose is determined, the patient is instructed to use this dosage and no more at the earliest sign of symptoms. It is important to emphasize shallow inhalations. Deep inhalations may lead to overdosage and side effects of severity since the drug is a powerful sympathomimetic.

On the basis of the obvious disparity in results of Norisodrine therapy in the comparatively mild asthmatics and the severe asthmatics, it can be postulated that the efficacy of the drug is based primarily on its bronchodilating effect. Conversely, it might be assumed that its effect on bronchomucosal edema is minimal. The foundation for these assumptions is, first, that bronchospasm is more apt to play a major role in the asthmatic attack where the condition is of long standing or great severity and, second, upon the laboratory demonstration of the marked broncho-dilating effect of the drug. In the early or mild asthmatic, mucosal edema is more apt to be the underlying mechanism of dyspnea and therefore, here, Norisodrine is less effective.

In summary, it can be said that Norisodrine is an unusually effective symptomatic therapeutic for severe asthma when inhaled as a dust. It

gives relief to epinephrine-fast asthmatics and those refractory to other anti-asthmatics. Under controlled conditions, there are few side-effects and little evidence of increasing tolerance.

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FLORIDA ALLERGY SOCIETY

We are very pleased to announce the formation of an allergy section of the Florida Medical Association known as the Florida Allergy Society. Officers for the present year are Dr. Clarence Bernstein of Orlando, President; Dr. Frederick Hieber of St. Petersburg, Vice-president and President-elect; and Dr. Nelson Zivitz of Miami Beach, Secretary-treasurer. Besides these officers, other charter members are Drs. Frank Metzger of Tampa, S. D. Klotz of Orlando, M. J. Flipse, Edwin D. Preston, James Putnam and Harold Rand of Miami, Louis Palay of Miami Beach, Frederick D. Droege of Sarasota, W. H. Gardner of West Palm Beach, J. M. McDonald of Jacksonville, and Claude Frazier, location pending. Dr. W. Ambrose McGee of Richmond, Virginia, was elected a new honorary member. At the organization meeting, April 23, Dr. S. D. Klotz presented a paper entitled "Allergy and the Heart," which was published in the May-June issue of ANNALS OF ALLERGY.

MODIFIED ANTIHISTAMINIC OINTMENT

Its Topical Use in the Treatment of Pruritus

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PRURITUS is the predominant subjective symptom in most cutaneous diseases. Unlike pain, its control cannot be satisfactorily accomplished by analgesics. Local applications which heighten the threshold of receptivity of the receptor organs of the skin ameliorate and often abolish pruritus. Also, for topical effect other mechanisms of action have been described. With the introduction of antihistaminic drugs there have become available a group of agents, the mechanism of action of which is believed to be a blocking of histamine elaboration, or a blocking of the combination of histamine with tissue cells. This has led several investigators to use antihistaminic drugs locally on pruritic eruptions. In such instances, their topical application has been shown to reduce the wheal reaction produced by application of histamine to the skin.[2] By local use, their benefits could be obtained without the untoward effects which often accompany oral administration (drowsiness, nausea, etc.). Among the investigators who have found the use of topical application of antihistamines satisfactory are Feinberg and Bernstein,[1] Orecklin,[3] Woolridge and Joseph,[5] and Sulzberger, Baer and Levin.[4] Most investigators have found them of value in circumscribed neurodermatitis and anogenital pruritus, as well as contact dermatitis due to poison ivy and insect bites.

On the theory that a combination of an antihistaminic drug with other agents believed to ameliorate pruritus may be productive of more rapid effect, there was selected by us for clinical trial an ointment* consisting of 2 per cent methapyriline hydrochloride with 10 per cent calmitol liquid** in a water-miscible base composed of stearyl alcohol, carbowax, sodium lauryl sulfates, and water.

DATA

Our clinical material consisted of seventy-five patients with various dermatoses, from private practice and from the out-patient clinic and dermatologic wards of Bellevue Hospital. Calthenamine ®, the modified antihistaminic ointment, was gently applied to the affected areas three times daily. Those patients exhibiting bilateral and symmetrical

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*This was supplied under its trade name CALTHENAMINE CREAM by Thomas Leeming & Co., Inc., New York.

**Calmitol liquid is composed of, per fld. oz.: Hyoscyamine Oleate 0.006 gr. (equiv. to Hyoscyamine alkaloid 0.003 gr.) chloral 1.29 gm., menthol 1.73 gm., camphor 1.64 gm., alcohol 14.3 c.c., ether 5.1 c.c. and chloroform 1.9 c.c.

lesions were also used as controls, a control ointment consisting purely of the water-miscible base and free of active ingredients being applied to the left side and Calthenamine to the right side.

The results were classified into three groups, i.e., *excellent, satisfactory,* and *failures.* In the *excellent* group were included those cases in which the response was both rapid and/or completely successful. In this group, the pruritus was mitigated or disappeared, sometimes in a few minutes, as in the case of insect bites. In some instances the pruritus disappeared with only one application and did not recur. In others, the relief lasted for a few hours, and upon reapplication of the ointment relief continued. In some patients in this *excellent* group, such as those with localized neurodermatitis (which is perpetuated by scratching), the cessation of pruritus led to rapid improvement of the lesions.

In the *satisfactory* group were included cases in which the pruritus was mitigated, but less consistently so, or for shorter periods than in the excellent group.

In some instances designated either as *excellent* or *satisfactory,* the itching recurred, since there was no amelioration of the dermatitis proper.

In the group of *failures* were included those patients who failed to respond to the modified antihistaminic ointment. In two cases the lesions were aggravated.

Urticaria.—Urticarias were divided into two groups: those due to insect bites and those due to other causes. Of six cases of insect bites, five were treated with excellent results and one failed to respond. In the successful cases the pruritus subsided, often within fifteen minutes of application. Of five cases of urticaria due to other causes (penicillin, physical allergy, etc.) one was treated with excellent results, three with satisfactory results, and one failed to respond.

Dermatitis Venenata (Contact Dermatitis).—This entity was divided into two groups. One group included contact dermatitis due to plants (poison ivy, etc.; the other included contact dermatitis due to substances other than plants. In the plant group, of eight cases of poison ivy dermatitis treated, five responded with excellent results and three failed to respond. These latter were acute cases in the vesicular stage. Those that responded favorably were cases in which the acute stage had subsided with wet dressings of boric acid or saline solution, for the treatment of the lesions proper as independent from the treatment of the pruritus. In dermatitis venenata of other than plant origin (the largest entity), the etiology of the eruptions and their location varied. In some instances the eruptions were due to contact with irritants handled in the patients' work; in others the causative agents were cleansing substances (soap, et cetera) or drugs. In a few cases, the offending agent could not be

determined. In this group of thirty-two patients with dermatitis venenata, eight responded excellently, fifteen satisfactorily, and nine were classed as failures.

Disseminate Neurodermatitis (Atopic Eczema).—Of eight patients treated, three obtained satisfactory relief from pruritus; pruritus recurred in two patients after a period of about two weeks. It is interesting to note that of the five failures, two obtained relief with Calthenamine base used as a control.

Localized Neurodermatitis.—Of five patients treated, two obtained excellent results: the pruritus completely disappeared and the lesions underwent rapid involution. In three the results were satisfactory, for the pruritus disappeared but the lesions were not modified. All lesions in this group were of long standing.

Anogenital Pruritus.—Of nine patients treated, only one excellent result was obtained; in four the results were satisfactory, and four failed to respond.

Miliaria (Prickly Heat).—Two patients with miliaria failed to respond.

Untoward Results.—In two cases, both of disseminate neurodermatitis (atopic eczema) dermatitis venenata was caused by the modified antihistaminic ointment. Application of the ointment to raw surfaces caused burning in some instances. Several patients with disseminate neurodermatitis reported a drying sensation after prolonged use of the ointment. Two cases of this group, with dry, lichenified skin, felt more comfortable by application of the control ointment than with the modified antihistaminic ointment.

SUMMARY AND CONCLUSIONS

1. A modified antihistaminic ointment (Calthenamine ® consisting of methapyrilene hydrochloride combined with calmitol liquid in a bland, water-miscible base) was investigated for the treatment of pruritus accompanying certain dermatological conditions. For control, the bland base of the same ointment was employed.

2. Excellent or satisfactory amelioration of pruritus was noted in most cases of urticaria from insect bites and other causes, localized neurodermatitis and dermatitis venenata. Fairly satisfactory relief of pruritus was noted in about half the cases of disseminate neurodermatitis and anogenital pruritus. Miliaria failed to respond. In two cases of disseminate neurodermatitis, the modified antihistaminic ointment increased irritation.

(Continued on Page 514)

PRECIPITIN REACTION IN THE DIAGNOSIS OF ALLERGIC PATIENTS

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IF the controversial but obvious relationship between the clinical phenomena of allergy and anaphylaxis is considered, it is no wonder that antibodies similar to those of anaphylaxis have been sought in the serum of patients with asthma, hay fever and other allergic diseases. The investigation of precipitins has been consistently negative until Cannon (cit. 1) and Cohen and Weller,[4] using Zozaya's method,[18] carried out a careful investigation of these antibodies in the serum of sensitized patients. The method rests upon the increase of the micella by adsorption of the antigen on collodion particles thus making precipitation easier. Cannon, besides finding precipitins in the sera of sensitized animals, found them in four of seven patients sensitized to egg; Cohen and Weller found them at low concentrations of up to 1/640 in a patient sensitized to fish-glue. In non-treated patients sensitized to ragweed, the results were always negative while six treated pollinosis patients exhibited weak results in two and strong in four.

Considering that this reaction is essentially precipitinic and only differs from those obtained with the usual techniques by its pronounced sensitivity to collodion, this fact was deemed to be important since from a conceptual viewpoint it confirms the similar mechanism between anaphylaxis and allergic shock. Having become acquainted with these papers we performed some works to show how often and under what conditions the precipitin reactions appear with this technique. Our clinical and experimental results have been collected in a series of papers which we have already published;[1,2,8,14] we shall now report them in a summarized form, laying stress on the practical and theoretical value which may be gained with this method of clinical examination in allergic disease.

To date, we have carried out the precipitin reactions in 2,400 cases grouped under different conditions in which allergic sensitization may be involved. One hundred and fifty-four normal subjects were used for control. Of the former cases, 755 belonged to asthmatic patients and in this report we shall only deal with them. A valuable experience has been gained from the painstaking studies carried out in these last four years which evidences the practical value—together with other methods—of precipitin studies in the determination of causative allergens. It is for this reason that a short report of the results have been set forth.

TECHNIQUE

The same technique described by Zozaya was used at first.[18] Collodion was well purified by washing in distilled water for six to ten days, then

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washed in 96 per cent alcohol thrice, twice in absolute alcohol and lastly dried with calcium chloride. Once purified, 5 grams were dissolved in 20 grams of absolute alcohol and in 75 grams of anhydrous ether. This solution is poured off, filtered and precipitated with distilled water, then washed several times and dried between sheets of filter paper. When dry, it is dissolved in very pure acetone, the solution is rapidly shaken in the electric shaker and distilled water is slowly added meanwhile to form a whitish suspension which is further diluted till clots appear. Acetone is excluded by vacuum distillation, and the large particles of collodion are removed from the remaining suspension by centrifuging at 3,000 r.p.m. for two minutes. Centrifuging is performed twice, once for ten minutes and another for thirty minutes (a few crystals of sodium chloride are added beforehand), thus separating the second and third fractions of the collodion particles. The latter, i.e. the finest, are washed with saline and kept in the icebox till use is made of them. Adsorption was then carried out with the antigen and the reaction was performed following Zozaya's method.

But Goodner's[7] procedure was soon made known to us, a simplified technique and therefore of greater use in practice. A series of comparative tests were performed with the same sera,[2] and it was noted that the latter technique was very exact. It was therefore put to current use with a few modifications. Antigen is poured into ten agglutination tests tubes (0.5 c.c. into each), diluted from 1/20 to 1/5120, and 0.1 c.c. of the patient's serum and 0.4 c.c. of the collodion suspension is added to each tube, prepared according to the technique of Zozaya. The concentration is the same as that of a 2,000 million germs per c.c. anti-typhoid vaccine. The tubes are shaken, taken to the autoclave for one hour at 37° C. and then kept in the icebox till the next day when the results are taken down.

Experience has taught a few facts which are considered interesting as regards practice. In the first place, old or hemolized sera must never be used. The former may give rise to non-specific precipitation if not perfectly kept, and as for hemoglobin in hemolized sera, it may likewise determine false reactions. It is deemed convenient that the subject be fasting when the blood sample is taken and that female patients should not be menstruating at the time. Otherwise, precipitations of doubtful value may be exhibited in some instances.

It is essential for the antigens to have a 7.8 pH, and this may be obtained with a phosphate buffer. The protein content is also of interest although it may not be the only substance which precipitates. When positive sera with different concentrations of antigen are examined, it is seen that the degree of the reaction varies according to the protein concentration of the antigen. Thus antigens are used with a known protein nitrogen content by adjusting the concentration in such a manner that 1 c.c. contains 10 mg. of protein. The antigen is diluted when the reaction is performed: 0.1 c.c. to 10 c.c. of saline, i.e., 1 mg. of protein in 10 c.c.

RESULTS

1. *Positive Reactions in Subjects with No Allergic Disease.*—With the precipitin reaction, positive results are obtained in 40 to 48 per cent of the allergic patients under study. This percentage varies somewhat in the different disease groups, but as a rule they fall within the above figures. The precipitin reactions have been studied in 120 subjects with no allergic disease and several of the most common food antigens have been tested, the same in these as in the other patients. One or more positive reactions were only obtained in fourteen cases (11.6 per cent). No positive results were obtained in thirty-four normal subjects tested with the precipitin reaction for different common airborne fungi.

This small proportion of positive results in normal subjects is all the more surprising when compared to the positive results in sensitized patients. It is a point in favor of the specific value of the positive results since, otherwise, a greater number of false positive results should be obtained in normal subjects.

The positive results in normal subjects do not of necessity indicate that they are non-specific since it is known that some subjects possess a symptomless sensitization. This is known as a balanced allergic condition (Vaughan;[16] Jimenez Diaz[9]). Passive transfer (Prausnitz-Küstner) may also elicit reagins in normal subjects (Tuft,[15] Colmer and Rackeman[11]). These facts only confirm the value of sensitization in many cases, to such a point that undeveloped sensitizations flare up when the patient contracts a condition which may be influenced by allergic shock, e.g., asthma or urticaria.

It may be gathered therefore that these reactions in normal subjects are rare and there is no reason for not considering them as specific, as though present in allergic patients.

2. *Precipitin Reactions in Patients Afflicted with Different Types of Bronchial Asthma.*—Our current experience involves 755 cases of asthma examined with this method. Of these, we have obtained one or several positive results in 307 cases to different antigens. Conversely, reactions were negative in 448 cases. The positive rate of 40.6 per cent contrasts with the 11.6 rate in non-allergic subjects.

Not all forms of asthma exhibit positive results at the same rate since, as we shall see presently, there are certain types of sensitization which never give a positive precipitin reaction, others only occasionally, and the remainder in which positive results are common.

(a) Pollinosis.—Thirty patients with pollinosis asthma have been investigated, using different pollen; in every instance, the cutaneous test and P-K.r. (Prausnitz-Küstner reaction) assured us of the sensitized condition. Our results have always been negative and it has therefore been inferred that no case of asthma by pollinosis is disclosed by precipitin reactions.

(b) Asthma influenced by food.—We have grouped the cases in which food is responsible for the asthmatic condition into two types: in one group those cases of anaphylactic asthma, or with pronounced idiosyncrasy, in which the onset of the asthmatic attack and other numberless phenomena (edema, urticaria, purpura, et cetera) is brought about by minute amounts of a foodstuff. It is the case of individuals sensitized to eggs, fish, et cetera, who mention the offensive food on being questioned. The other group is made up of cases which are more common, involving several foods and therefore more complex and not always brought to the patient's notice. It is the complex food asthma (Jimenez Diaz;[9] Funk[6]) or intrinsic asthma according to Rackeman.[12] The difficulties encountered in practice to establish the different factors involved is well known by all. It is for this reason that elimination diets have been used, such as those of Rowe[13] or the leukopenic test of Vaughan[17] or pulse count as advocated by Coca.[3] In our experience, only the elimination diet can furnish reliable data in some cases although, in many others, it is useless and, furthermore, it is a tedious and complicated method at times.

It is in this type of patient that interest lies in a diagnostic method which renders food influence objective since cutaneous reactions are negative and weak or multiple results are of little value. Passive transfer is almost always negative, and for this reason Coca[3] has termed this type of allergy "familial non-reaginic food-allergy" or idioblapsis. The fact is that it is precisely in this group that we have obtained the most interesting results with the precipitin reactions.

Of 328 cases of complex food asthma, 144 exhibited positive precipitins using ten antigens which are those used commonly as a starting point.

Positive results are distributed in the manner shown in Table I. The percentages refer to each food in particular, that is to say, to the number of times it has been positive. It must be remembered that many patients exhibited positive reactions to two or more foods.

TABLE I.—FREQUENCY OF POSITIVE RESULTS FOR EACH FOOD

Bread	27 times=18.7%	White-fish	19 times=13.1%
Potato	19 times=13.1%	Blue-fish	30 times=20.8%
Banana	12 times= 8.3%	Meat	56 times=38.8%
Rice	12 times= 8.3%	Milk	19 time=13.1%
Shell fish	23 times=15.9%	Eggs	36 times=25 %

The practical value of these positive reactions is shown in the first place by the therapeutical results obtained by excluding harmful foods in the diet of the patient. In the group of asthmatic subjects we have noted favorable results in 60 per cent of positive cases. The results range from cases of cure to others of simple improvements of symptoms, according to the role played by food sensitization, alone or together with other factors, in the etiology of the asthmatic picture.

Although the arguments which uphold the specific value of precipitin reactions shall be discussed later on, we shall set forth a few examples to show our point.

Enr. Mor.—Male, thirty-six years (No. 823). Ever since the age of twenty-five, attacks of asthma which begin with stoppage of the nose, hydrorrhoea and sneezing. In the last years, they are more common and pronounced. On some days he is afflicted with four to six attacks during night and day time. Precipitin reaction elicits sensitization to potatoes, eggs, milk and white-fish. Elimination diets are prescribed on these data and the patient improves considerably. When he eats one of the above-mentioned foods, he is overcome by wheezing and oppressiveness in the chest fifteen minutes afterwards.

Mar. Rod.—Female, thirty-five years (No. 849). Complex asthma with major symptoms since childhood. All the findings are negative excepting the precipitin reaction which is positive for the kidney bean, blue vetch, bread, large bean and lentil. After a few months on an elimination diet, the patient reports that she is in excellent condition and that she has suffered no attacks.

These cases involve subjects with a non-pronounced type of food sensitization, who are seldom aware of the kind of harmful foods involved. We identify these conditions with Coca's non-reaginic allergy. On the other hand, in cases of marked anaphylactic sensitizations, the patient is usually aware of the harmful foods. Precipitins can be detected with the standard methods (named macroprecipitins by ourselves), while precipitins (microprecipitins) elicited with the collodion technique are negative.

J. Cab.—Male, twenty-two years (No. 2400). Eczema in face and head since childhood, which lasted for a long time. Eating potatoes, eggs and milk gave rise to highly pruriginous wheals and angioneurotic edema of the face and lips. Simultaneously, long-lasting catarrh and asthmatic bronchitis. At the age of thirteen, being in the country during harvest time, he had an attack of asthma which lasted for several hours. Since then, asthmatic crises from time to time which appear whenever he eats eggs or hake. Macroprecipitins are positive up to ¼ dilution of antigen for egg or hake. Precipitins with the collodion technique (microprecipitins) are negative with the same offending foods (antigen dilutions begin at 1/10).

TABLE II. POSITIVE FREQUENCY FOR EACH FUNGUS IN THE 58 POSITIVE CASES

Mucor	27 times=46.7%	Botrytis	10 times=17.2%
Alternaria	25 times=43.1%	Cladosporium	4 times= 6.8%
Aspergillus	25 times=39.6%	Sterigmatocistitis	4 times= 5.1%
Penicillium	18 times=31.0%	Macrosporium	2 times= 3.4%
Stysanopsis	10 times=17.2%		

(c) Asthma due to fungi.—In the group of asthma due to sensitiveness to air-borne fungi, our present experience involves 160 cases studied with the precipitin reaction. At first, we only performed precipitation investigation in individuals who exhibited positive intracutaneous reactions to one or several of the tested fungi. Thus, in the first series of ten patients with clear sensitivity in the cutaneous tests, three were found with positive precipitin reactions to the very same fungi *(Alternaria, Aspergillus, Penicillium)* which had given rise to positive cutaneous reactions. More recently we have also tested the precipitin technique in cases of asthma which, on account of the clinical characteristics (connection with seacoast climate, time of onset, et cetera) seemingly involved fungi. Nevertheless, cutaneous reactions were negative. Thus do we find that of 160 total cases studied, fifty-eight had precipitins in the plasma involving one or several proven fungi (36.2 per cent positive).

Positive frequency for each fungus is shown in Table II in which percentages are reckoned in the same way as for the foods.

It is worth while to compare the behavior of positive precipitin reactions with the results of cutaneous tests, as shown in Table III.

TABLE III. COMPARISON BETWEEN POSITIVE PRECIPITIN REACTIONS AND THE RESULTS OF THE CUTANEOUS TESTS

	Pos. Precip. Pos. Intracut.	Pos. Precip. Neg. Intracut.	Total
Mucor	10	17	27
Alternaria	7	18	25
Aspergillus	7	16	23
Penicillium	5	13	18
Stysanopsis	4	6	10
Botrytis	4	6	10
Cladosporium	2	2	4
Sterigmatocistitis	1	2	3
Macrosporium	0	2	2

At first, agreement between both tests was a source of great worry to us since it was considered that the existence of precipitins in the patient's serum confirmed the existence of reagins in the skin; we admitted the possibility that it might be the same antibody evidenced by different routes. Later on, wide clinical and experimental experience reported in different publications[1,2,8,14] has convinced us that the precipitin (microprecipitin) and reagin are two different antibodies which may or may not coexist in the same subject. It is to be noted that, in the above Table III cases exhibiting agreement between both tests are fewer than those exhibiting disagreement, i.e., in which a positive precipitin reaction is noted with negative cutaneous reactions. This fact is very significant since objective data—the precipitins—are elicited in many cases of asthma suspected of being produced by sensitivity to fungi and yet showing negative results with the classical tests. This contention is true to such a point that in our department of allergy, wherever a patient is examined with a clear history involving a climatic factor and showing a positive precipitin reaction to one or several fungi although the cutaneous reactions are negative, we prescribe a desensitizing treatment with extracts of the offending fungi. We can thus treat a great number of patients with specific means who would otherwise be treated with non-specific measures. The therapeutical results gained in these instances support this procedure.

The following cases are given as demonstrative examples of the facts contended above:

May. Berg.—Female, eight years (No. 2430). Since the age of three, asthmatic attacks with great frequency which disappear whenever she leaves the seacoast town where she is living. Among the many treatments prescribed, the last has been penicillin aerosol. On inhaling the drug for the first time, she is overcome by an acute shock involving spasm of the glottis, apnea, cyanosis, et cetera, reduced after a time with injections of adrenalin. The cutaneous reactions and P-K.r. are positive to *Penicillium* and penicillin. Precipitin test is strongly positive for penicillin and still more for *Penicillium*.

In this case, the cutaneous reactions and precipitins were positive and the latter confirmed the value of the first. The following case is even more interesting since only the precipitins were positive:

Ad. Nog.—Male, nineteen years (No 3303). Six months ago, bronchitis with large amounts of sputum; fever ranging from 38° to 39° C. Dyspnea and coughing more recently, particularly at night-time. Dampness troubles him, giving rise to fatigue and hoarseness. Cutaneous reactions are negative to different antigens, among them *Mucor, Alternaria, Cladosporium* and *Aspergillus.* Nevertheless, precipitins are strongly positive with aspergillus. The patient is treated with three concentrations of *Aspergillus* extract. He is once again examined six months later and found to be in excellent condition, without fatigue or cough and having a good appetite.

(d) Asthma produced by cereal dust and parasitic fungi.—In asthmatic patients sensitized to dried vegetable or cereal dust and to parasitic fungi of the latter (tilletia, ustilago), it is common to elicit positive cutaneous reactions and passive transfer of sensitiveness with P-K.r. In many cases, determination of precipitins with the collodion technique shows positive results with the same antigens, agreeing therefore with the cutaneous tests. But in other cases, in which the clinical history evidences sensitiveness to cereal dust or to parasitic fungi, the cutaneous tests are negative. In these instances, the same as in asthma due to air-borne fungi, the precipitin reaction is a valuable aid in the etiologic diagnosis of the sensitization.

We have studied 182 patients with asthma who, on being questioned, gave details which pointed to sensitization by dried vegetable or cereal dust, owing to the fact that onset befalls when in contact with granary dust, straw, et cetera. The presence of precipitins was disclosed in eighty-four cases with suspected antigens (46.1 per cent), coincidental or not with positive cutaneous tests.

The number of times that each of the tested antigens was positive and the behaviour of cutaneous reactions is shown in Table IV.

TABLE IV. ASTHMA DUE TO DRIED VEGETABLES, CEREALS AND PARASITIC FUNGI: FREQUENCY OF POSITIVE PRECIPITINS AND COMPARISON WITH CUTANEOUS TESTS

Antigen	No. Times Tested	No. Times Pos. Reaction	Per cent Pos. Reaction	No. Times Pos. Intracut.	Per cent Pos. Intracut.
Tilletia	86	33	38.3	14	19
Ustilago	86	23	26.7	8	15
Cereal dust	90	27	30.0	11	16
Wheat	62	12	19.3	4	8
Barley	50	10	20.0	4	6
Rye	48	10	20.8	5	5
Oats	22	5	22.7	3	2
Tare dust	27	8	29.6	7	1
Indian bean dust	3	1		1	0
E. ervilia dust	1	1		1	0
Blue vetch dust	2	1		1	0

With this series of patients, something similar to what occurs in the group of asthma due to fungi is found to exist; the rate of negative cutaneous tests and positive precipitin reactions is greater than the number of times both tests are found to agree. It is once more stressed that the reason for this lies in the different existing antibodies which may or may not be found simultaneously in a patient. In many cases, both tests comple-

ment each other since the cutaneous reaction showed that one antigen was the sensitizing agent while the precipitin test elicited another. Hence, patients have been successfully treated who otherwise would have entailed failure. It can be affirmed on studying our statistics that the detection of precipitins is a better guide to treatment than positive cutaneous reactions since it is common for the latter to be negative with no evident passive transfer (P-K.r.). The following examples stress our point:

Gab. Gag.—Female, forty-five years (No. 2882). Lives in the country. Four months ago, dyspnea, wheezing and oppresiveness in chest, frequently recurring. Smoke and home and granary dust trouble her. When she came to Madrid, she became symptomless. Cutaneous tests with airborne fungi, parasitic fungi, home and cereal dust were negative. Precipitins positive with cereal dust. She is treated with cereal extract becoming symptom-free.

Hip. Ru.—Male, fifty-one years (No. 3190). Tends to be afflicted with catarrh. Twelve years ago, during one of these bouts, pronounced fatigue which lasted for four days. From then on and coincident with catarrh, dyspnea and wheezing lasting for several days. Occasionally, attacks with underlying asthma. He is greatly troubled by smoke, and cereal, wheat, barley and rye dust. Precipitins positive on wheat and rye test. Cutaneous tests negative for all the antigens tested. Shows striking improvement on wheat and rye extract treatment.

Pet. Zorr.—Female, forty-seven years (No. 3208). At the age of twenty-six, pronounced catarrh involving fatigue and bouts of dyspnea at night. Since then, has a common tendency to catarrhs and fatigue. When breathing air carrying granary, straw or threshing floor dust, he is overcome by pronounced fatigue and wheezing. Serum tests elicit strongly positive precipitins for tilletia, rye and oats. Conversely, cutaneous reactions are all negative. Shows great improvement on treatment with extracts of these offending antigens.

Precipitins occasionally elicit the existence of reagins when cutaneous reactions are negative (possibly due to a defective technique or to a weak antigenic power of the extract) or when values are non-specific.

Sant. Tom.—Female, thirty-four years (No. 2820). Nine years ago, acute catarrh with wheezing, dyspnea and thoracic oppressiveness involving hydrorrhea of the nose and photophobia. Remained in good health for three years and then exhibited attacks of asthma. Since then, coryza and fatigue which increases when moving wheat to the granary. Cannot feed the cattle because he is overcome by breathlessness. Precipitins positive for ustilago and cereal dust. Cutaneous reactions are negative with these antigens. Nevertheless, when results of precipitin reactions were known, passive transfer (P-K.r.) was performed and clear positive results were obtained for ustilago and cereal dust.

Alej. Par.—Female, fifty-eight years (No. 3279). Catarrh every winter with fatigue and wheezing. In June and July her condition worsens. Cereal dust makes sneeze and brings on fatigue. Cutaneous reactions: tilletia, negative; ustilago, + + +. Precipitins: tilletia, + ++ ++ +; ustilago, negative. P-K.r.: tilletia, + + +; ustilago, negative.

(e) Other types of asthma.—Different cases of asthma due to varying agents have been collected in this group. The precipitin technique following our procedure was of great diagnostic value in some instances.

Animal fluff (epithelial scales) and other skin products: Nine cases have been studied in which sensitization by fluff of different animals was suspected. Cutaneous reactions were positive for all these antigens but precipitins were only positive in five cases.

Eduv. Rui.—Female, forty-four years (No. 1385). From the age of twenty, asthmatic attacks, mainly at night. They only supervene in her native village and her condition improves when traveling. She cannot go into a stable since this act brings on pronounced dyspnea. Precipitins positive for mule fluff. Intracutaneous and P-K. reactions positive. Irritation test (nasal reaction) is likewise positive.

The following is an unusual case of sensitiveness to cat hair. Cutaneous and precipitin reactions were clearly positive.

Mar. Fra.—Female, eighteen years (No. 1282). Attacks of asthma since a child. During the intervals between attacks, fatigue of varying degree. Numberless tests (cutaneous) with different antigens were negative. It was discovered that two cats slept in her bed. Cat hair extract was used to investigate precipitins in the patient's serum. Results were highly positive as also the cutaneous reactions which involved pronounced local and general phenomena. The cats were removed from her home and her condition improved leaving her symptomless.

Precipitins occurred in four cases of twelve involving asthma due to woolen materials or feathers. One of these is particularly interesting since sensitiveness to the wool of the mattress was suspected but cutaneous tests were negative.

Merc. Gab.—Female, twenty-nine years (No. 2481). Since childhood, attacks of asthma which have increased in number and intensity. Onset usually supervenes in bed at night. If she goes to bed during daytime, she is also afflicted by asthmatic attacks. The mattress contains woolen material. Cold and dampness worsen her condition. Cutaneous reactions using wool from her mattress are negative. Precipitin with the same antigen are positive. Treatment is prescribed with three strengths of wool extract. Four months latter she writes, ". . . . I have followed treatment according to your instructions and I am now free from fatigue. I occasionally feel slight disturbances and cough a bit at night. . . ."

In nine cases, precipitins for dust from the home of the asthmatic patient were investigated. Results were positive in three cases; likewise the cutaneous tests.

In twelve cases of asthma due to insects, we have never obtained precipitins in those due to cimex, whereas cutaneous reactions and P-K.r. were clearly positive. There were ten cases of cimex sensitization. The two remaining cases were due to the wheat weevil ("Calandra granarius" or "sitophilus granarius") and in these, precipitins were positive. The first case has already been published (Jimenez Diaz, Lahoz and Canto).[10]

Within the group of occupational asthmas, we have collected twelve cases. Precipitins were studied. Five patients were bakers, four millers and the other three handled different kinds of flour. Positive precipitins were found in six cases: three for common flour, two for flour containing parasites, and one for mill dust.

Man. Can.—Male, forty-five years (No. 3407). Baker. For many years he tends to be afflicted with catarrh, wheezing and oppression in the chest. In the last twelve months, attacks with underlying asthma, particularly during sleep (he sleeps during the day) and when in the bakery. Flour dust troubles him greatly. He feels much better whenever he leaves work. Cutaneous reactions are negative for many antigens including several kinds of flour. Precipitins are clearly positive for parasitic flour (wheat).

This and many other cases prove clearly that a correct diagnosis of the offending and sensitizing antigen would never have been established if the precipitin reaction had not been used. It exemplifies the value of this technique, particularly where cutaneous tests remain negative.

In Table V, all the cases of asthma mentioned in the last group have been collected.

TABLE V

	Total	Posit. Precip.	Nega. Precip.	Pos. Precip. Pos. Intracut.	Pos. Precip. Neg. Intracut.
Animal fluff	9	5	4	5	0
Cat hair	1	1	0	1	0
Wool and feathers	12	4	8	2	2
Dust from the patient's home	9	3	6	1	2
Insects: Cimex	10	0	10	0	0
Insects: Calandra	2	2	0	2	0
Occupational asthma	12	6	6	2	4
	55	21	34	13	8

It can be affirmed, on glossing over the foregoing, that the precipitin reaction is useless in pollinosis asthma, since sensitization in this type of allergy is purely reaginic.

In non-anaphylactic food asthma, in which it is considered that cutaneous tests are of no value and other tests are unreliable (Coca's and Vaughan's tests), precipitin determination is held to be the best for diagnosis (microprecipitin sensitization).[1,8,14]

The following are included in both groups: climatic asthma, asthma due to dried vegetable dust, to cereal, house, animal fluff, insect dusts (excluding cimex) et cetera. In these, precipitins furnish, in some cases, confirmatory proof of sensitization elicited by cutaneous tests and, in other cases, they complement the results of the latter. Lastly, in numerous cases, they elicit sensitizations which would otherwise have gone unperceived.

TABLE VI. SUMMARY OF CASES OF ASTHMA STUDIED WITH THE PRECIPITIN TECHNIQUE

Group	Number	Pos. Precip.	Per Cent	Neg. Precip.	Pos. Precip. Pos. Intracut.	Pos. Precip. Neg. Intracut.
Pollinosis asthma	30	0	= 0	30	—	—
Food asthma	328	144	=43.9	184	—	—
Fungi asthma	160	58	=36.2	102	30	84
Cereal asthma, etc.	182	84	=46.1	98	59	77
Several others	55	21	=38.1	34	13	8
Total	755	307	=40.6	448	102	169

DISCUSSION

It is deemed that the data put forth in this paper show the great practical

interest attached to the precipitin reactions, as performed by ourselves, in routine and systematic examination of asthmatic patients.

Doubt might be expressed as to the specific value of these reactions. The following arguments are set forth as a proof of clinical value and specific character of this method:

1. The reactions obtained in many of these patients have been repeatedly carried out. Where the patients have not been treated meanwhile, the results have been uniform. It is contended that if the reactions were not specific, the results would vary from case to case.

2. The results of the tests and the case history have always been coincidental. Positive reactions have never been obtained with cereal extract in patients who have never come into contact with them. The same hold for home dust, fungi, et cetera. However, on testing food, fourteen positive tests have been obtained in 120 subjects who were not asthmatic and who were not afflicted with any other allergic condition. However, this fact does not discredit the method since it involves symptomless sensitization.

3. In numerous cases in which precipitin reactions have been positive, stimulation tests have been confirmatory.

4. The elimination of the offending antigen has influenced the course of the condition. When the precipitin reaction is only positive for one antigen in a patient, e.g., cases of asthma due to dust, suppression of contact has done away with the attacks.

5. Another point in favor of the specific value of the precipitins evidenced by collodion technique is the experimental and elective production in laboratory animals; reports have been published elsewhere by ourselves.[1,8]

The specific value of this test is deemed to be evident. The reason for sensitiveness with positive precipitin reaction and others with negative results remains to be discussed. It is believed that the same occurs in this instance as with cutaneous reactions and passive transfer. There are sensitized patients who exhibit positive reactions with the latter while others exhibit negative results. When pollen causes asthma, cutaneous reactions and passive transfer tests are commonly positive but, as mentioned beforehand, precipitins are never noted. Conversely, when food sensitiveness is considered—excepting marked idiosyncrasy in cases of complex, multiple sensitization—cutaneous reaction and P-K.r. are negative since this type of sensitiveness involves non-reaginic components. It is obvious that the precipitin antibody evidenced by this technique differs from the reagin and may or may not be coincidental. Hence, reaginic, precipitinic or mixed sensitiveness is found. It probably depends on the nature of the antigen, route of entry and degree of contact. The experimental studies performed[1,8,14] confirm these viewpoints.

It has been deemed essential to find out whether the antibody which causes these reactions is the precipitin obtained experimentally in sensitized animals. A series of studies have shown that such is not the case. In animals with positive precipitins according to the classical technique, which

can be termed macrotechnique, it is found that the diluted serum will not exhibit precipitin reactions performed with the collodion technique, which may be termed microtechnique. The same holds true for appropriate sera of patients. For example, no positive reactions are obtained with collodion when the serum of a patient with precipitins (macrotechnique) for strawberries is diluted. Thus, it is believed that the causative antibody in these reactions is not the anaphylactic precipitin nor is it connected to the reagin.

Investigations have also been carried out to see if the blocking-antibody of Cooke, Bernard, Heball and Stull[5] might be involved. For this reason, microprecipitins in the sera of patients under treatment for pollinosis and blocking-antibody titres have been studied at the same time. Results were negative: blocking antibodies rose steadily but the microprecipitin reaction was consistently negative.

In short, we are dealing with a little-known antibody which we have termed microprecipitin for the time being, heat-stable,[14] the offending antigen for a certain time.[1,8,14] It is definitely not the common precipitin (macroprecipitin), reagin or blocking-antibody. There is evidently a macroprecipitinic or anaphylactoid sensitization, as evidenced by experimental anaphylaxis or major idiosyncrasy in man, generally caused by a certain food. A reaginic sensitization undoubtedly exists, best exemplified by pollinosis, with reagins and without microprecipitins. Lastly, we have sensitizations (many cases of multiple food sensitiveness, dusts, fungi, parasites, et cetera) with microprecipitins which may or may not associate with reagins.

SUMMARY

The authors describe the technique for the precipitin reaction, based upon the adsorption of the antigen on collodion. It has been used to study the existence of specific antibodies in normal subjects and in asthmatic patients where etiology involves different factors. This antibody, termed microprecipitin by the authors, bears no connection to the precipitin shown with the classical technique, nor to the reagin or blocking-antibody.

In the serum of patients with certain types of allergy, a precipitin of the anaphylactic type can be demonstrated, in others, the reagin and, in others still, the microprecipitin. Thus the authors admit three types of allergy: macroprecipitinic, reaginic and microprecipitinic. As a rule, in major sensitizations (idiosyncrasy or anaphylaxis due to food), macroprecipitins and reagins are demonstrated. Only reagins are found in pollinosis and in complex, non-reaginic, food sensitization, in which neither the cutaneous reaction nor the passive transfer are positive, microprecitins are found. In asthmatic patients sensitized to different kinds of dust, in climatic asthma and in those due to danders and insects, both antibodies —microprecipitin and reagin—or only one of them can be found. Data on

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FOOD ALLERGY

A General Discussion of Twenty-five Years of Experience

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THIS discussion is based on some twenty-five years of a practice limited to allergic diseases, which naturally would include the study of many, probably several hundred, cases in which food allergy played the sole or a significant role.

The purpose of this discussion is to clarify some practical points that are of importance in reducing failures in the management of these cases. These points to be mentioned are based on our own difficulties and are based also on cases that have actually been only too frequently overlooked by internists and allergists who had previously handled many of these cases. The overlooking of these points brings unjustified discredit to methods which are highly successful if properly applied.

The first practical point is that while conditions such as infantile eczema, adult atopic dermatitis, and allergic cephalalgias can be perhaps usually ascribed to food sensitivity, such is not by any means invariably the case. An appreciable number of cases of eczemas especially fall into the category of contact dermatitis in which the food element plays only a minor or absolutely no part whatsoever. Even chronic urticaria is not invariably due to food allergy. We have in our records a few such instances due to bedding and the usual house dust factors. The pollen element enters into a small number of urticaria cases, proven by the cessation of cutaneous lesions on unrestricted diet on removal to an atmospheric pollen free environment. Many cases of migraine or allergic headaches have been reported by others due purely to antigens of the house dust type. Nocturnal or early morning symptomatology with nasal blockage definitely suggests a possible non-dietetic etiology.

Thus the failure to secure positive skin tests to foods in all instances where expected, should lead not to unfavorable criticisms of the tests, but to consideration of non-dietary factors. This statement would also include naturally those cases where the withdrawal of secured positive skin test foods has made no change in the clinical picture. These positive reactions may represent long forgotten past sensitivities. They also could indicate current clinical sensitivity only with rare excessive ingestion, or act under certain conditions, as factors in the production of allergic symptoms entirely foreign to those presented at the time being. However, they may be of decided importance taken in conjunction with the main non-dietary factors, or the main non-reacting foods. Their routine withdrawal if they are not too numerous does no harm, even if only of occasional necessity. Consequently, in the above mentioned conditions the finding of negative skin tests to foods may be entirely correct.

Now a few more words regarding skin tests. In spite of many apparently incongruous findings, I feel that skin testing is extremely valuable and should be a routine procedure. I find the dermal tests seldom of diagnostic value. However, they should be done in considerable number for two reasons: first as a preliminary index of safety for subsequent intradermal work, and secondly, to exclude the possibility of some unusual unsuspected antigen, occasionally of a high degree of sensitivity, such as flaxseed or cottonseed.

Some consideration here should be given to the not uncommon failure to secure the expected definite whealing intradermal tests to food antigens later proven of clinical significance.

First comes the masking of each reaction by antihistaminic drugs, a matter of increasing importance with the prevailing widespread self and ordered administration of such drugs. Secondly comes the fact that in many such ingestion cases due to commonly used foods such as milk and the cereals, the actual degree of sensitivity is low, beyond the powers of the skin tests to produce typical diagnostic wheals, giving as a test result merely small papules. A child taking two quarts of milk a day with only chronic indigestion and general ill being, must possess actually only a relatively low degree of sensitivity. Were such sensitivity of high degree calling for a positive scratch test or usual undoubted diagnostic intradermal wheal, the clinical ingestion response would be immediate vomiting or symptoms of acute severity.

In these instances of low degree sensitivity, the intradermally secured small papule or a delayed tuberculin type reaction may thus be diagnostically correct.

Also, certain testing materials such as strawberries frequently give negative skin tests in the face of undoubted clinical sensitivity.

Another complicating factor is that false positives are occasionally secured in deteriorated testing material, probably from some decomposing histaminic element.

Still another disconcerting factor consists of the numerous false positives seen in the urticarial hypersensitive skin, making specific diagnosis by skin tests impossible. Passive transfer here may be resorted to but is rarely necessary, as such extreme skin hypersensitivity will usually disappear after a few weeks or less employment of a diet later to be suggested.

In routine testing in food allergy cases where there is no cutaneous irritability, one or several positives, usually of a minor degree, are encountered fairly frequently. These may or may not be of clinical significance. However, these minor positives should be closely regarded during the active symptom period, in spite of the fact that they may be only temporary in character and of no importance following symptom clearing. However, one reason for failure in this work is the assigning of primary importance to these minor accessory food factors with weak positive

skin tests, and the exclusion from consideration of the actual prime specific non-dietetic factors. Food positives in bronchial asthma fall into this category. We only occasionally find any dietetic item of clinical importance in the production of asthma.

Where specific food allergy has been correctly diagnosed and where the appropriate corrective dietary measures have been instituted, another cause of failure is the non-recognition of the fact that in old chronic cases following initial betterment a period of time, possibly of several weeks, may be required before complete elimination of the pathological process occurs; especially is this true of old chronic cereal urticaria or eczema cases. This occasionally leads to the partial or total abandonment of the absolutely needed prolonged strict cereal abstention, and the unwarranted assumption of psychomatic influences, or of the importance of the other food positives found on the initial or at various later test sittings. This state of affairs has also been noticed by Albert H. Rowe of Oakland, California.[2]

Another common cause of failure in food allergy is the non-recognition of the deleterious effects of drugs (other than the antihistamines), especially those of a purgative laxative or analgesic character, also opiates and salicylate derivatives such as aspirin. Constipation should be corrected where necessary by low enemas, not by drugs. Occasionally a few drops of dilute hydrochloric acid daily will assist in reducing flatulence where present. On the whole, it is safe to recommend complete elimination of drugs. The antihistaminic drugs, of course, do not fall into this category. They could and should be used to the degree needed for comfort, especially in skin cases, to obviate delayed resolution due to non-specific irritation from itching and scratching.

Another extremely important point now presents itself. It is undoubtedly true that a single cereal sensitivity at times exists. However, in my experience, this is far from being the usual state of affairs, and the non-recognition of wheat sensitivity in many instances as a complete cereal group sensitivity is an extremely frequent cause of failure in wheat and cereal cases. For consistent results, I have not been able to substitute any one cereal for another, and hence all are omitted, also of course, all cereal products (bread, biscuit, crackers, hot cakes, waffles, spaghetti, flour thickened soups and gravies). Malted drinks, corn, rice and rye products are included in this prohibited category. Our experience shows that many failures are due to the use of breads and wafers of these substitute cereal products in wheat cases. The rice content explains the many failures of the long used diagnostic semi-starvation diet of lamb, rice and canned pears. Well-done crisp melba toast made from plain, not whole wheat flour, oven heated for twenty-five to thirty minutes, makes an excellent cheap practical bread substitute. In twenty-five years we have encountered only a single cereal case that could not tolerate melba toast

so prepared. Hence we find totally unnecessary the more expensive and more difficult to prepare, bean and potato flour substitutes. The Mexican corn tortilla crisply toasted makes another excellent bread substitute.

NAME_____ DATE From To

BREAKFAST	LUNCHEON	DINNER	EXTRAS	SYMPTOMS
Sunday				
Monday				
Tuesday				
Wednesday				
Thursday				
Friday				
Saturday				

Fig. 1

Another rarer cause of failure exists. I am referring to the patients' other allergic conditions such as vasomotor rhinitis or pollenosis. Their correction by appropriate measures may be needed in some instances to assist in restoring the allergy balance necessary for the clearing of an obstinate food allergy affecting primarily the skin or gastrointestinal tract.

Local dietary habits are possibly of some importance in the relative frequency of individual antigenic food items. For instance, in southwest Texas, citrus fruits are of importance and we seldom see fish or potato cases.

An understanding of these points is necessary before any degree of consistently good results can be expected from any form of dietary control of food allergy.

Specific diagnosis in food allergy can be determined by various methods:

1. By overfeeding of suspected foods.
2. By skin tests.
3. By dietary restrictions and subsequent resumption of individual food items.

The first method of overfeeding suspected food elements to intentionally increase symptoms has not attracted our office, and we have had very little experience with it. It will occasionally differentiate between contact and dietary eczematous conditions.

Some form of dietary diary is essential in this work. One with a column giving the hour of symptom onset is especially valuable. A careful scrutiny of this dietary diary and repeated testing of the possible incriminating deleterious factors it suggests, will usually sooner or later reveal in primary milk or cereal cases, secondary unsuspected sensitivity or minor sensitivities that are delaying clearing (Fig. 1).

The appended diet that follows, reported by us in 1940,[1] has proven its value by its routine use in our office for many years, a diet which has cleared a high percentage of old chronic cases within a few weeks, and even, in many instances, within a few days before the completion of food testing, which in our office is routinely spread out to several, not necessarily daily, sittings. This is due to the fact that a very high percentage of chronic food allergy is due to milk and the cereals which the diet takes care of. This diet gives these patients a practical diet of some variety, comparatively cheap, easily followed, and with the advantage of advising definitely what to eat as well as what to avoid. Of course, it is only a basic diet and will not uncover fruit, vegetable, or the more unusual sensitivities. These should be picked up by repeated testing and studying of the dietary diary.

SUGGESTED BASIC DIET FOR FOOD ALLERGY CASES

Breakfast.—Citrus fruit not more than once weekly. Other mornings, apples peeled or cooked, stewed prunes, grapes or grape juice. Crisp bacon, well cooked eggs, coffee or tea.

Dinner and Supper.—All meats, well cooked, except pork. All fowl. All white meat, scale fish.

All cooked vegetables (Watch tomato). Lettuce or cooked vegetable salads.

Barred: Milk (as a beverage) nuts, chocolate, shell fish, berries, honey, melons, Coca Cola and all soda fountain drinks, and condiments except salt. Wheat and all cereal products except melba toast. This prohibition includes bread, biscuit, crackers, macaroni, spaghetti, cakes, flour-thickened gravies or soups, beer and in fact all malted drinks. Melba toast crumbs makes a good batter material. Tapioca, sago, and arrowroot where available may make good cereal substitutes. A small amount of accompanying canned milk is not forbidden. Potato flour can usually be substituted for thickening soups or gravies. Bread made from it we have not found practical. At least initially, total abstention from alcohol is preferable. If this is impossible to secure, the Mexican tequila or brandies derived from fruits make fairly good substitutes for grain derived alcoholic beverages.

In acute cases, or where dietary restrictions are to be of limited time duration, raw foods of every character are omitted. In this connection, in acute urticaria our own experience shows the common practice of an initial laxative or purgative apparently more harmful than beneficial.

Production of symptoms by ingestion of incompatible foods on an empty stomach is of course a requisite from a scientific proof point of view. Practically, it is not a necessary routine procedure. Patients will often through inadvertent, unavoidable, or intentional ingestion furnish such proof even where most of the time the food is not taken on an empty stomach.

However, as a matter of fact, no matter how desirable it may be from a confirmatory scientific point of view, it is not always possible to secure such confirmation of correctly diagnosed offending foods by dietary resumption on an empty stomach. Patients will not always consent. Also, even if instituted, the procedure is not invariably successful for the reason that it is not always possible to reproduce all the conditions specific and non-specific, which initiate the symptomatology. This is especially true if the sensitivities are multiple.

Patients are advised in advance of the weight loss that ordinarily follows any extended use of this basic diet. However, there is no loss of strength, and occupational activities ordinarily need not be restricted. Fattening foods can be added later, as shown innocuous by tests and clinical trial.

Neither our office nor our patients like permanent abstention from comparatively essential foods such as milk, eggs, or the cereals. With milk cases, except in very young children, we have no difficulties. We encounter very few adult cases desirous of its resumption. The various milk substitutes needed in pediatric practice are beyond the province of this discussion. However, canned milk will frequently be found innocuous in definite raw milk-sensitive cases, making a good cheap substitute. Curi-

ously enough, we see very few egg cases. Practically all of our egg cases have no difficulty with hard cooked eggs. In the rare cases of extreme egg sensitivity, of course, meticulous total abstention is required.

With our cereal cases what our patients miss most is bread. Ordinarily, the sensitivity of wheat cases is not of extreme degree. By starting with an eighth of a slice of plain white bread and by long continued daily use, and slow increases over a period of weeks or months, we are able either to produce a certain amount of hyposensitization or to establish or discover a tolerance permitting a fairly satisfactory usage. We find this more practical than the flour-water method.

The acute cases of food allergy are usually self-limited and seldom offer any difficulties in specific diagnoses or handling. Testing is seldom called for.

The subacute and chronic cases present an entirely different problem of vastly greater difficulty. A simple practical basic initial diet for the control of these cases is presented. However, no conclusions should be drawn as to the value or nonvalue of this or any other dietary regime for the control of food allergy without a full understanding and consideration of the introductory factors mentioned at some length.

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MODIFIED ANTIHISTAMINIC OINTMENT

(Continued from Page 495)

3. None of the patients obtained relief of pruritus from the control ointment except two cases of disseminate neurodermatitis.

4. Although the investigation was essentially directed at a determination of the effect of the modified antihistaminic ointment in the control of pruritus, it was noted that in many of the successful cases, an improvement in the appearance of the lesions took place. The mechanism may have been one wherein the combination of histamine and tissue cells was prevented, or one wherein with the amelioration of pruritus (reducing the need for scratching) the lesions improved.

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EXPERIMENTAL AND CLINICAL EFFICACY OF TRIMETON AND CHLOR-TRIMETON MALEATE

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THE discovery by French scientists (Bovet, Staub, Halpern and Fourneau) that certain derivatives of ethylenediamine and ethanolamine are antagonists of histamine provided the impetus for extensive research resulting in the synthesis of many chemically related antihistaminic agents. Investigations in the laboratories of Schering Corporation have shown that an entirely new class of compounds, the propylamines, exhibits outstanding antihistaminic activity. Fortunately, these compounds are relatively nontoxic; and, when these laboratory findings were confirmed in the clinic, two new antihistamines, prophenpyridamine (Trimeton) and chlorprophenpyridamine maleate (Chlor-Trimeton Maleate) were introduced.[7,17,18]

The antihistaminic activity of drugs can be evaluated by observing the inhibition of the pharmacodynamic effects of histamine. Among various *in vitro* and *in vivo* methods for the assay of antihistaminic activity, the most reliable and widely employed one is an *in vivo* test which measures the degree of protection of guinea pigs treated with antihistamines and challenged with lethal amounts of histamine (Halpern,[5] LaBelle and Tislow,[7] Loew[9]).

Guinea pigs are very sensitive to histamine. After administration of lethal doses of histamine, they exhibit signs of respiratory distress within a few minutes; convulsions and death occur. The mechanism involved is a muscular spasm of the bronchioles, which results in asphyxiation. However, guinea pigs which have received a sufficiently large oral or parenteral dose of an antihistamine prior to a lethal dose of histamine are protected from the bronchial spasm. The size of the dose of an antihistamine necessary to provide this protection is a measure of its antihistaminic activity.

At least three graded doses of the various antihistamines were administered orally to guinea pigs. Exactly one hour later each animal was challenged intravenously with a lethal dose of histamine dihydrochloride (1.1 mg/Kg of body weight). The number of protected animals was recorded, and the median protective dose (PD_{50}) with confidence limits was calculated by the method of Litchfield and Wilcoxon.[8]

In the acute toxicity determinations, several graded doses of the antihistamines were given orally to guinea pigs. The mortality was recorded for five days after feeding the drug. The median lethal dose (LD_{50}) and confidence limits were then calculated.

From the Schering Corporation, Bloomfield, New Jersey.

TABLE I. ORAL ACTIVITY AND TOXICITY OF SEVERAL ANTIHISTAMINES IN GUINEA PIGS

Compound	Median Protective Dose		Relative Potency	Median Lethal Dose		Therapeutic Index LD50/PD50	Relative Safety
	PD50	Confidence Limits*		LD50	Confidence Limits*		
	mg/Kg	mg/Kg		mg/Kg	mg/Kg		
Chlor-Trimeton (chlorprophenpyridamine maleate)	0.14	0.11 to 0.17	100.0	210	175 to 250	1500	100.0
Trimeton (prophenpyridamine)	1.5	1.1 to 2.0	9.3	270	218 to 321	180	12.0
Neo-Antergan (pyranisamine maleate)	2.5	1.6 to 3.8	5.6	290	221 to 380	116	7.7
Histadyl (thenylpyramine—HCl)	4.0	1.8 to 8.8	3.5	460	336 to 632	115	7.7
Benadryl (diphenhydramine—HCl)	4.0	3.5 to 4.5	3.5	280	200 to 390	70	4.7
Thephorin (phenindamine)	3.2	2.7 to 3.9	4.4	185	124 to 276	58	3.9
Pyribenzamine (tripelennamine—HCl)	3.2	2.6 to 3.9	4.4	150	110 to 200	47	3.1
Neohetramine (thonzylamine—HCl)	14.5	10.4 to 20.3	1.0	370	283 to 485	26	1.7
Antistine (phenazoline—HCl)	36.0	25.7 to 50.4	0.4	440	306 to 633	12	0.8

*P—0.05

DISCUSSION

Statements have been made in a recent publication by Dreyer[3] on the comparative properties of certain antihistaminic agents, although no quantitative data were presented to substantiate such statements. The comments based on certain *in vitro* procedures performed on guinea pig ileum, guinea pig uterus, isolated frog heart and isolated guinea pig heart do not present an objective evaluation of the activity of the drugs. Likewise, no data are reported on *in vivo* tests which would permit quantitative evaluation of drug efficacy. Dreyer alluded to the Lovejoy, Feinberg and Canterbury[10] histamine flare test in man, and proceeds to cite his results of capillary permeability tests in rabbits. He neglects to state that Lovejoy, Feinberg and Canterbury in their tests on man report Trimeton to be distinctly more active than Neohetramine.

Analysis of Dreyer's blood pressure experiments with histamine reveals that this test does not adequately differentiate the efficacy of antihistamines. At one point, Dreyer suggests that thonzylamine is a hypotensive agent, whereas there is an implication that prophenpyridamine is pressor; yet elsewhere in the text the author distinctly states that prophenpyridamine, in common with other antihistamines, has both pressor and hypotensive properties dependent upon the dosage administered.

As shown in Table I, Chlor-Trimeton Maleate is clearly outstanding in that it has the greatest activity of the large series of antihistamines studied.* Since the acute toxicity of Chlor-Trimeton Maleate is comparable to that of the other agents, its therapeutic index and relative safety are the highest

*We have since tested every new antihistamine as it became available and still find Chlor-Trimeton Maleate to be by far the most active.

TABLE II. COMPARATIVE EXPERIMENTAL AND CLINICAL ACTIVITY OF SEVERAL ANTIHISTAMINES

Antihistaminic Drugs	Relative Index of Activity in the Guinea Pig	Clinically* Effective Dose in Man	Relative Index of Clinical Activity
		mg/70 Kg	
Chlor-Trimeton (chlorprophenpyridamine maleate)	100.0	2 to 4	100.0
Trimeton (prophenpyridamine)	9.3	10 to 25	17.0
Neo-Antergan (pyranisamine maleate)	5.6	25 to 50	8.0
Histadyl (thenylpyramine—HCl)	3.5	50 to 100	4.0
Benadryl (diphenhydramine—HCl)	3.5	25 to 50	8.0
Thephorin (phenindamine)	4.4	25 to 50	8.0
Pyribenzamine (tripelennamine—HCl)	4.4	25 to 50	8.0
Neohetramine (thonzylamine—HCl)	1.0	25 to 100	4.8
Antistine (phenazoline—HCl)	0.4	50 to 100	4.0

*Based on dosage recommendations in published clinical reports.

in the group. These values are 60 times the corresponding ones for Neohetramine, and 125 times those of Antistine.

Dreyer administered 50 mg/Kg of Chlor-Trimeton Maleate to guinea pigs, which represents *333* times the oral effective dose 50 without untoward effect, and this confirms the remarkable safety of the drug. In contrast, if one administers as little as *30* times the oral effective dose 50 of Neohetramine, 50 to 65 per cent of the guinea pigs die according to oral toxicity data on Neohetramine as published by Reinhard and Scudi,[14] and this is confirmed by our own data (Table I).

The outstanding safety of Trimeton and Chlor-Trimeton Maleate has been further verified by extensive chronic toxicity studies in rats and dogs. Lifetime oral toxicity tests[18] in rats conducted for three generations and similar studies in dogs continued for two generations have revealed the absence of any toxic or cumulative effects.

The validity of the animal experiments summarized in Table I is verified by the remarkable parallelism between the experimental and clinical activities of several antihistamines as shown in Table II. Based on quantitative experimental data, Trimeton has been shown to have superior efficacy and safety as compared with the older antihistamines. Extensive clinical research by and the experiences of numerous physicians confirm this superiority.[2,6,11,12,13,15,16,20,21] Chlor-Trimeton Maleate is the most effective of all antihistamines and has the highest degree of safety of those evaluated to date. These laboratory findings regarding Chlor-Trimeton Maleate have been borne out by clinical reports.[1,4,19,22]

SUMMARY

A review of the literature shows a remarkable parallelism between the therapeutic indices obtained in the laboratory and the excellent clinical

results obtained with Trimeton (prophenpyridamine) and Chlor-Trimeton Maleate (chlorprophenpyridamine maleate).

Using guinea pigs as test animals, oral toxicity and antihistaminic activity tests were performed on nine commonly used antihistamines. The therapeutic indices were: Chlor-Trimeton Maleate, 1,500; Trimeton, 180; Neo-Antergan, 116; Histadyl, 115; Benadryl, 70; Thephorin, 58; Pyribenzamine, 47; Neohetramine, 26; and Antistine, 12.

All these findings may be attributed to the unique chemical structures of Trimeton and Chlor-Trimeton Maleate.

Experimentally the relative safety of Chlor-Trimeton Maleate and of Trimeton is greater than any of the other drugs tested.

The relatively greater safety and efficacy of Chlor-Trimeton Maleate and Trimeton by comparison with other antihistamines as indicated in the laboratory have been borne out by numerous clinical reports.

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ALLERGY TO SO-CALLED "INERT INGREDIENTS" (EXCIPIENTS) OF PHARMACEUTICAL PREPARATIONS

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AN excipient, according to Webster's Dictionary, is "an inert substance used in preparing remedies in order to give them a suitable form or consistency."

The major excipients used for binding the ingredients of pills, tablets, et cetera, include corn starch, milk sugar, gum acacia, gum tragacanth, licorice powder, flour, sugar and various chemicals.

Additional potentially allergenic materials employed in pharmacy in the manufacture of tablets, powders and capsules, as granulating agents, lubricating and disintegrating agents, flavorings and ingredients of coatings and capsules, include glucose, pectin, agar, gelatin, honey, gum karaya, casein derivatives, cocoa butter, chocolate, fresh eggs, corn oil, soy bean oil and olive oil.

During the past three years inquiries have been made of the principal manufacturers of encapsulated vitamins, and the following list of materials pertinent in this connection have been named as occurring in the capsule or filling of such medications: corn starch, corn sugar (glucose), granulated sugar, "wheat product," pork gelatin, acacia, beeswax, corn oil, cottonseed oil, soy bean oil, peanut oil and sesame oil.

Flavoring agents employed in prescription writing include such food items as syrup of cacao, raspberry, cherry, orange, rhubarb, cinnamon and sarsaparilla.

Non-aqueous vehicles for injectable medicinals include gelatin and oils of peanut, sesame and almond.

Manufacturing processes of official preparations not infrequently employ allergenic food stuffs or their derivatives. For instance, the current pharmacopoeia (XIII) permits the use of liquid glucose (derived from corn) and malt preparations (derived from barley and which, as a rule, are allergenic for the wheat-sensitive patient) as solid extracts. Corn starch, lactose, sucrose and powdered licorice (glycyrrhiza) are permitted as powdered extracts. Tinctures and alcoholic extracts of drugs may prove to be allergenic in certain instances, as the alcohol is usually derived from grain and it is recognized that cereal-sensitive patients are frequently intolerant to grain alcohol. To our knowledge the allergenicity of pure samples of alcohol has not been carefully studied in known cases of sensitivity to corn, wheat, malt, et cetera.

It immediately becomes apparent that these lists, although by no means complete, contain many common allergenic foods. Furthermore the manu-

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facturing pharmacists are under no legal or other obligation to list such ingredients on the labels of their products. The Food and Drug Administration is interested only in the active ingredients of medicinal preparations, there being no stipulation regarding the labeling of excipients and diluents, many of which are of considerable interest to the allergist.

The most frequently encountered clinical problems in dealing with pharmaceutical preparations for allergic patients occur with those individuals highly sensitive to corn or milk.

CORN AS AN EXCIPIENT

Corn starch is the most widely used of all inert ingredients in pharmacy. A physician is safe in assuming, at least until disproved by the statement of the manufacturer, that corn starch is present in all tablets designed for ingestion.* The first instance of the allergenicity of corn employed as an excipient and diluent was reported by Rinkel[19] in 1944, although he[20] had made the observation in 1936 that the ingestion of corn contained in a proprietary medication employed for the relief of coughing actually increased this symptom. The patient, known to be corn sensitive, failed to respond to corn avoidance until he ceased taking this medication. Readministration of the preparation resulted in the recurrence of allergic symptoms, shown unequivocally to have been due to the corn content of the medication.

Case E. R., a woman, aged fifty-four, previously reported in brief,[13] had been subject to severe intermittent headaches for fifteen years and status migrainous for the past decade. This had been associated with constant dizziness to the point that she was unable to leave her home for months at a time and on many days had been unable to walk. When first seen she also complained of a debilitating degree of weakness, alternating constipation and diarrhea and a chronic dermatitis of her hands.

Cutaneous and intracutaneous tests with inhalants revealed that she was sensitive to house dust. She was shown to have a widespread allergic response to foods as indicated by the precipitation of several acute clinical reactions following individual food tests (Rinkel's[21] technique as confirmed by Randolph and Rawling[14]). Corn proved to be one of her most troublesome food allergens. With the maintenance of dust therapy, the complete avoidance of corn in addition to other incriminated foods and the rotation of tolerated foods in her diet, her allergic symptoms were effectively relieved for a period of several months, except as she inadvertently encountered a source of one of her food allergens. This was accomplished in the absence of all medication.

Then, for reasons unexplained at the time, she noticed a gradual recurrence of her chronic fatigue, followed in turn by dull constant headaches. It was first thought that her known food allergens had not been completely eliminated but sources of exposure could not be detected. It was then assumed that she had developed a sen-

*A simple test to determine the presence of starch in tablets or powders consists of applying a weak solution of potassium iodide or tincture of iodine. The presence of starch is indicated by the development of a bluish, purple color. It should be emphasized that this test is not specific for corn starch but a positive reaction is presumptive evidence that it is present in a preparation unlabeled in respect to excipients in view of the fact that it is by all means the most common vegetable starch employed for this purpose. Flour, tapioca or arrowroot starch, to name others occasionally employed as excipients, will also give a positive iodine reaction.

One is sometimes aided in identifying a given type of starch by the microscopic appearance of the individual granules. Excellent illustrative plates exist in many older texts.[8,10]

sitivity to a new food, but all questionable items in the diet, as revealed by her food diary, were checked by performing individual food tests and found to be compatible. By a fortuitous circumstance, she omitted her tablet of desiccated thyroid for a period of four days; this medication had been re-prescribed two months earlier because of the suggestive symptoms of hypothyroidism and the finding of a basal metabolism of minus 16 per cent. However, when it had been taken prior to the establishment of her allergic diagnosis, it had failed to alter the course of her chronic fatigue or other symptoms. With omission of this tablet, she felt immeasurably improved by the end of the third day.

The same tablet was then reintroduced at noon on the fifth day of avoidance and was followed by marked yawning and somnolence. She lapsed into a stuporous sleep forty-five minutes after taking the tablet and continued to sleep all the afternoon and evening. Residual fatigue persisted the following day. The tablet in question gave a positive iodine test for starch and was found to contain corn starch as an excipient, on the statement of the manufacturer.

The patient was then placed on desiccated thyroid free of corn starch and other excipients commonly associated with allergic reactions** and has tolerated this preparation for the past year.

On another occasion she had a gradual return of her fatigue and headache shortly after starting to take tablets from an old supply of ferrum reductum.

One other time when hospitalized for the surgical removal of a papilloma, she was given aspirin for the relief of local pain and within an hour complained of an excruciating headache.

She has tolerated ferric ammonium citrate and corn-free aspirin for many months. Several different times she has been given tablets of thyroid or aspirin "blindly" without knowledge as to whether or not they contained corn starch, and on each occasion she was able to detect the presence of the corn starch excipient as a result of her symptom reaction. In the numerous times this was tried, not once did she incriminate the presence of corn products when they did not exist in the test tablet.

Case M. A., a woman, aged twenty-nine, had complained of the symptoms of chronic nasal allergy, intermittent headaches associated with myalgia of the posterior cervical muscles,[15] childhood and fall hay fever for the past three years, complicated by seasonal asthma.

She was found clinically sensitive to house dust and several fall pollens for which she was specifically treated.

Because of the history of noticing itching of her nose when ironing, she was immediately suspected of being corn sensitive and prepared for an individual food test. Although she had her usual continuous mild headache prior to the experimental feeding of corn meal and corn sugar, she noticed a sharp accentuation of the head pain in association with somnolence and rhinorrhea beginning five minutes after the trial ingestion. Within an hour she developed a severe chill, remaining chilly and complaining of generalized aching throughout the remainder of the afternoon. This clinical reaction, simulating the grippe, required four days to subside.

With continuation of specific inhalant therapy and the avoidance of corn and other proven food allergens, she had no troublesome symptoms until the onset of the 1947 ragweed pollen season, at which time she developed moderately severe hay fever. A well known antihistaminic drug was prescribed in the hope of diminishing her symptoms, but she became worse immediately after taking each tablet. After a few days of this therapy she started to have severe and constant asthma in addition to hay

**A line of common pharmaceuticals available in tablet form excluding corn and other major allergenic excipients is available from the Upjohn Company, Kalamazoo 99, Michigan, under the trade name, "Abergic."

Of the antihistaminic drugs, Benadryl in the form of the elixir and 50 mg. kapseal (Parke, Davis), Pyrrolazote (Upjohn), Hydryllin (Searles) and Histadyl (Lilly) are known to be currently free of the major allergenic excipients.

fever and a recurrence of her troublesome headaches. Her symptoms promptly improved with the cessation of the drug in spite of continuing high ragweed pollen counts. The same medication was started again after four days of avoidance; this was again followed by severe headaches and coughing. At this point it was ascertained from the manufacturer that the tablet contained corn starch as an excipient.

The pharmaceutical firm kindly made available the antihistaminic drug in crystalline form; this as well as the crushed tablets were placed in colored capsules and administered to the patient experimentally without her knowledge of which contained the excipient. She developed a recurrence of her coughing and headache only following ingestion of the preparation containing corn.

One other corn-sensitive patient, allegedly sensitive to sulfadiazine, in that its administration was invariably followed by rhinitis and coughing, has been able to tolerate the Abergic brand without untoward effects.

Several other corn-sensitive individuals on a regimen of avoiding corn and products derived from it have commented spontaneously on the greater effectiveness of corn-free aspirin in relieving their aches and pains in comparison with the standard commercial product.

Observations bearing on the allergenicity of corn sugar (glucose and dextrose) have recently been presented.[16] The writer has seen two highly corn-sensitive patients in which the use of lozenges was suspected of causing a recurrence of their symptoms of the type due to corn sensitivity. In each instance, the lozenges were found to contain corn sugar.

Corn oil, in the amount ordinarily employed in pharmaceutical preparations, has not been proved to cause symptoms in corn-sensitive patients. However, certain corn-sensitive individuals have been observed to develop symptoms following the experimental or accidental ingestion of corn oil. The oil appears to be the least allergenic of all the corn products.

MILK AS AN EXCIPIENT

Speaking generally, one may assume that tablets designed to be dissolved for hypodermic injection contain lactose or milk sugar. Because of its relatively low deliquescence, milk sugar is the favorite diluent to give bulk to powders and to mix with powdered medicinals for filling capsules. That lactose is capable of eliciting allergic symptoms in highly milk-sensitive individuals is attested by the following cases:

Case M. W., a woman, aged thirty-one, had been subject to espisodes of nausea, vomiting and diarrhea for eight years; severe fatigue and generalized muscle soreness and headache for three years; and unexplained urgency and frequency of urination for three months. She had been receiving frequent hypodermic injections of codeine for relief of her headaches and cervical myalgia. For a period of several months she had noticed a progressively severe degree of local pruritus at the sites of her codeine injections.

In the avoidance of milk in preparation for an individual food test, she was changed from the standard codeine prepared from tablets containing milk sugar to a solution of codeine crystals dissolved in saline. She promptly commented on the absence of itching at the sites of her injections and on the greater effectiveness of the drug in relieving her pain and aching, although she was receiving a constant dose.

An individual food test with milk was followed in eight minutes by the onset of sharp epigastric colicky pain which radiated through to mid-back. A second feeding of milk an hour later was associated with a sharp accentuation of her previous pain. Her gastrointestinal reaction persisted through the night and for a period of three hours was associated with generalized pruritus. All symptoms had subsided by the following morning except for residual abdominal soreness.

With the complete avoidance of milk and numerous other incriminated foods, she has had complete relief of her pruritus and has shown marked improvement of other symptoms. For a period of several weeks during her diagnostic studies she remained subject to intermittent headaches for which codeine was administered. During this interval she was treated both with lactose-containing codeine and milk-free codeine without a knowledge of which type she was receiving. Invariably, she was able to state whether or not the injection contained lactose as a result of the associated local pruritus and the effectiveness of the analgesic action.

Parallel subcutaneous and parallel intradermal injections of the varieties of the drug failed to reveal any significant differences in the relative sizes of the local reactions, but in each instance she was able to identify the injection containing lactose by the presence of intense local pruritus.

The case of I. K., a nurse, aged twenty-three, subject to chronic incapacitating headaches, has been previously reported.[17] She was found to have an exceedingly high degree of milk sensitivity. On several occasions the inhalation of the fumes of cooking milk (osmyls) was sufficient exposure to produce an acute allergic response.

Shortly after her original diagnosis was made, the inadvertent ingestion of small amounts of milk or butter would be followed by the sudden onset of a violent headache. She learned that such attacks could be materially reduced in severity if a hypodermic injection of codeine was administered with the first warning of the episode, but codeine was not nearly as effective if she waited until a needle and syringe could be sterilized. Consequently, she was supplied with a rubber stoppered vial of codeine solution containing crystals of codeine phosphate dissolved in normal saline. She not only experienced no abnormal local reaction from these injections but obtained the customary analgesic and sedative actions from the drug.

At this point (that is, at the time her case was reported), she moved to another section of the country and very soon began having a recurrence of her chronic headaches for which she continued to take codeine. She began noticing a gradual change in its action in that the injections became relatively ineffective in relieving her head pain, and instead of obtaining the expected sedative action from the drug, she noticed that it seemed to make her more tense, irritable and excited. She obtained transitory and partial relief of her headache immediately following an injection but then her head pain would become more severe and the more codeine she took the more excited she became. She had also developed very large local reactions from the injections.

It then developed that she had been using a codeine solution prepared from tablets rather than crystals. Without informing the patient that codeine tablets usually contain lactose as an excipient, an identical dose of the drug prepared from crystals dissolved in saline solution was administered. She subsequently commented, spontaneously, that she had obtained more relief of her pain from that injection than from any other she had received for two years and immediately inquired as to how it had differed. She further observed that for the first time in months she did not have a local reaction from the injection.

In view of the extreme severity of her reaction to milk, she was not subjected to additional experimentation with codeine preparations containing lactose.

GUM TRAGACANTH AS AN EXCIPIENT

Brown and Crepea[1] recently presented a case of asthma and urticaria due to gum tragacanth employed as an excipient in a well known antihistaminic tablet. They suggested that the minor ingredients of a given pharmaceutical preparation should be carefully investigated before attributing sensitivity reactions to the principal ingredient. Allergic reactions from the inhalation of gum tragacanth particles have been emphasized by Gelfand.[9]

GUM ACACIA

To our knowledge, gum acacia or gum arabic has not been incriminated as a cause of allergic reactions as an excipient in acacia-sensitive individuals, but Spielman and Baldwin[24] have pointed out that acacia is commonly used as an emulsifying agent in the preparation of pharmaceutical preparations. The allergenicity of acacia has also been emphasized by Maytum and Magath,[12] Studdeford,[26] Levin,[11] and Feinberg and Schoenkerman.[5]

GUM KARAYA

Neither has gum karaya been specifically incriminated as a cause of allergic reactions from its use as an excipient in medicinal tablets. Karaya sensitivity, first reported by Bullen[3] and confirmed by Feinberg,[6] Bowen[2] and Figley,[7] is contained in many laxatives and dentifrices.

LICORICE (GLYCYRRHIZA)

Although instances cannot be found where glycyrrhiza has been reported to cause allergic symptoms from its occasional use as an excipient, it is known to sensitize and this possibility should be kept in mind. The author has a case of sensitivity to licorice in a patient highly sensitive to other legumes.

OTHER SUGARS

The allergenicity of corn sugar (dextrose and glucose) and milk sugar (lactose) has previously been discussed.

In the writer's experience, beet sensitivity is common in areas where beet sugar is used as the principal source of sugar and is not an infrequent allergen in other regions. The ingestion of granulated beet sugar will cause symptoms in many beet-sensitive patients, confirming the experience of Rinkel[22] and Zindler.[28] The use of beet sugar in the simple syrup of prescriptions has resulted in the production of allergic symptoms in patients known to be beet sensitive, whereas the same prescriptions were tolerated in the absence of beet sugar.

Sensitivity to cane was first emphasized by Coca.[4] Although less common than beet sensitivity, it is being recognized with increasing frequency since it has been suspected as a cause of symptoms in all undiagnosed patients and tested by means of the individual food test technique[14,21]

Several instances have been proved as a result of this diagnostic approach. Prescriptions containing cane sugar in the form of simple syrup have been shown to cause allergic symptoms in these individuals when all other ingredients of the prescriptions were test negative.

The practice of using sucrose in simple syrups, regardless of its origin, makes it necessary for physicians dealing with patients allergic to one or more sugars to stipulate in writing the prescription the type of sucrose that he desires to be employed.

GELATIN AS AN EXCIPIENT

Contrary to the alleged non-antigenicity of gelatin,[25,27] Rinkel[23] has observed several instances of allergic reactions to the ingestion of gelatin in beef- and pork-sensitive patients. The writer[18] recently reported clinical reactions to the ingestion of commercial gelatin in three of four highly beef-sensitive individuals.

The patients were diagnosed specifically by means of individual food tests with beef, and in each instance severe allergic symptoms followed the experimental feeding of this food. They were then tested by means of the individual food test technique with one quarter ounce of gelatine (Knox) dissolved in warm water. Three of the four developed unmistakable symptoms immediately following the test feeding; in each instance the symptom response was similar to that associated with the original individual food test with beef. A week later three of the same patients ingested 150 ml. of gelatine prepared for intravenous use (Knox), and two of the four (the same two who had reacted to the ingestion of commercial gelatin) also developed unmistakable symptoms.

In additional unpublished observations, the above patient with the most marked reaction to the ingestion of gelatin prepared for intravenous use was retested with the ingestion of a similar amount of another brand of beef gelatin at a time when she was still exquisitively sensitive, as judged by the fact that she reacted with clinical symptoms from eating pork cut on a beef butcher block. Ossine gelatin (Wilson) prepared for pharmaceutical purposes failed to produce a clinical reaction. Another exceedingly pork-sensitive individual, as judged by the development of rhinitis and migraine on several occasions following the inhalation of cooking pork, also failed to develop a demonstrable symptom response after the ingestion of pork gelatin (Wilson) prepared for the pharmaceutical trade.

In summary, it may be said that at least certain brands of gelatin may cause symptoms in the highly beef-or pork-sensitive patient. This would appear not to be invariably true, and individual cases should be studied in their possible reaction to individual preparations.

Instances have not been observed by the writer where gelatin employed as an excipient has caused reactions in known beef- or pork-sensitive patients.

DISCUSSION

It is generally recognized that the absolute avoidance of specific foods is an essential requirement both in the diagnosis and treatment of allergic individuals highly sensitive to those foods, otherwise the specific diagnosis may be in error and the treatment ineffective. For example, if one diagnoses food allergy by means of individual food tests, a prime requirement for interpretation of such tests is that the food in question be avoided for a short period prior to the experimental feeding. If one employs restricted diets in diagnosis, relief of symptoms may not ensue if a given allergenic food is continued to be ingested in the form of an excipient.

Numerous instances have been cited in which, although the diagnosis had been correct, the incomplete avoidance of the allergen in question due to its continued ingestion as an excipient, failed to result in the expected amelioration of clinical symptoms. This course of events is apt to lead one to the false conclusion that the specific diagnosis is incomplete and not infrequently results in an unnecessary prolongation of diagnostic studies. Furthermore, and from the long range standpoint, one cannot expect the degree of sensitivity to a particular food to subside significantly, even with the avoidance of dietary sources, if it is received several times daily in medicinal sources.

Inasmuch as it is frequently desirable and sometimes necessary to continue the use of medications during periods of specific diagnosis and therapy, it behooves the clinician in this field to become familiar with the excipients employed in the medications that he is accustomed to using. The only way in which this may be accomplished at present is by the cumbersome procedure of addressing inquiries directly to the manufacturing pharmacists.

Even though a physician is familiar with the food stuffs that he is prescribing in medications, it frequently becomes a difficult task to protect his patient from specific exposures. The problem can be handled reasonably satisfactorily where all medications are prescribed by a single individual solely responsible for the patient's health. Difficulties are apt to arise in instances where this responsibility is shared with other medical colleagues, and, particularly, if the patient is inclined to indulge in self-medication.

The greatest hazard experienced to date has been in handling the allergic patient who requires hospitalization. Simply ordering a corn-free regimen, for instance, is not sufficient, due to the fact that interns and hospital pharmacists are ordinarily not familiar with the distribution of excipients in pharmaceutical preparations, and, more importantly, neither have ready access to this data. Although a given intern may be made familiar with this problem, such precautions are apt to break down when he is off duty or when he is succeeded by a fellow house officer.

This emphasis on medications as food and, for that matter, even the restriction of specific food allergens, has no parallel in the diagnosis and

treatment of other medical conditions encountered in the general hospital. The infinite care to accomplish such a program does not fit harmoniously with other hospital tasks. As a consequence, blunders from the standpoint of the specific elimination of foods are very apt to occur, even though the physician in charge attempts to anticipate all such possibilities. A striking example of this type of error may be cited. A patient with sufficiently severe gastrointestinal symptoms to require hospitalization was shown to be sensitive to milk, as evidenced by the relief of his digestive disturbances following the avoidance of this food and the reproduction of distressing symptoms in association with its experimental ingestion. Explicit orders were written proscribing all dairy products and limiting medications to those known to be free of lactose. Furthermore, the patient was fully aware of the deleterious effects of milk and had been thoroughly briefed in the sources and possible ways in which he might encounter this food.

On making his evening rounds, the writer was confronted with an angry, violently ill patient who had previously been recovering satisfactorily. A milk and molasses enema had been ordered by the intern, the order had been checked by the floor nurse, then assigned to and executed by the orderly. Within the first few minutes of this "treatment," the patient complained bitterly of pain, withdrew the tube and demanded to know what he had been given. This unfortunate experience precipitated several days of acute illness and unnecessarily prolonged his hospitalization. He was finally persuaded not to bring suit against the institution.

The present chaotic condition in respect to the recognition of the clinical importance of excipients is exemplified by the fact that one popular antihistaminic drug, even though designed for the use of allergy patients, contains milk sugar in one size dosage but does not contain this excipient in the other. It is also revealing that neither the Food and Drug Administration nor the editors of the Pharmacopoeia have made any effort to regulate this aspect of drug manufacture, referring to the food products under discussion merely as "inert ingredients." Descriptions of accepted products by the Council of Pharmacy and Chemistry of the American Medical Association for inclusion in New and Non-official Remedies do not contain this information even though it has been supplied by the manufacturer.

The purpose of this presentation is not to injure any particular preparation or pharmaceutical firm but to call to the attention of the medical profession, the Council of Pharmacy and Chemistry of the American Medical Association, the manufacturing and dispensing pharmacists, the Food and Drug Administrator and the editors of the United States Pharmacopoeia, that the physicians dealing with food-sensitive allergic patients not only need to be informed but have the right to know the food ingredients of the medications that they are prescribing. It is suggested that pharmaceutical firms change their excipients and binders to less allergenic substances and that they state the composition of the "inert" as well as the active ingredients of their preparations.

SUMMARY

Various food products, designated as "inert ingredients," are employed as excipients or binders in the manufacturing processes of practically all tablet medications and in many other forms of pharmaceutical preparations. Neither by common practice nor current regulation are these food ingredients labeled on the package.

Certain patients, highly sensitive to such common foods as corn and milk for instance, may have allergic symptoms precipitated by the ingestion or injection of medicinals containing these specific excipients.

Illustrative cases are cited.

Addendum: Four patients highly sensitive to corn have been observed to have symptoms identical to those produced by the test ingestion of corn or corn products after the administration of penicillin G.

A reaction consisting of severe headache with nuchal myalgia, nausea, vomiting and diarrhea occurred each of four times in one adult; a child age seven developed abdominal cramps and an elevation in temperature to 101.0 degrees F. on each of two occasions and a child three years of age developed severe diarrhea after each attempt to administer penicillin G by injection. Penicillin in oil or aqueous form produced these responses. Another adult developed an acute rhinitis following the use of penicillin G in the form of troches which also contained dextrose of corn origin.

The fungus, penicillium, from which penicillin is harvested, is usually grown on corn steep liquor. In as much as the first three patients have tolerated penicillin O (prepared by the Upjohn Company, Kalamazoo, Michigan, from non-corn nutrient sources and not containing additional corn in the form of excipients or diluents) when administered by injection and the fourth has tolerated corn-free penicillin O troches, it is strongly suggested that penicillin as ordinarily available commercially carries the allergenicity of the corn steep liquor, upon which it is grown, into the manufactured product.

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PRECIPITIN REACTION

(Continued from Page 507)

statistics and examples of the different groups are shown, stressing the practical value of microprecipitin determination in diagnosis of allergic disease.

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COMPARING THE IRRITANT ACTION OF SOAPS

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SOAPS are the oldest, most universally used, and, all things considered, the most satisfactory means of removing soil from the normal human skin. They are the first of the synthetic detergents.

There are various theories as to how soaps remove soil from the skin, the most commonly accepted of which is the one that soap cleans because of its power to emulsify and suspend dirt particles in such form that they may be floated off the skin. The soap molecule consists of a water-soluble end (the alkali) and an oil-soluble end (the fatty acid radical). When a soap solution contacts dirt particles the soap molecules surround the particles, each with its oil-soluble end turned so that it is in contact with the dirt particle. The water-soluble ends project out into the solution. The dirt particle is in effect floated by a multitude of adhering soap molecules and is thus prevented from settling out of the soap solution or redepositing on the surface.

Soaps, by their action of removing soil from the skin, also have a secondary deodorant action because they remove from the skin substances which give off offensive odors. Substances which give off offensive odors may consist of (1) foreign matter deposited on the skin, (2) secretions of the skin glands which have decomposed on the skin, (3) abnormal secretions of the skin glands having an offensive odor.

Soaps, as well as other skin cleansers, not only act on the secretions of the skin and soil deposited on the skin, but also have a definite action on the skin itself. The alkaline solution tends to soften, loosen and dissolve the keratin layers of the skin. Not only do soaps emulsify, dissolve and remove foreign oils, but they tend to remove the natural oils, fats and waxes from the skin, thus tending to drying, thinning and shrivelling of the skin. Hence the prolonged action of soaps may result in dermatitis, especially in the case of persons having naturally dry skins. Therefore, the prolonged immersion of the skin in low concentrations of soaps or exposures of the skin for shorter periods to strong concentrations of soaps and other skin cleansers may result in a dermatitis due to primary irritation. In addition to this, some skins may become allergic to alkalis or to certain fatty acids and their salts. Certain soaps are more likely to irritate the skin than others, as for instance it is generally recognized that potassium soaps are more likely to irritate the skin than sodium soaps, and that soaps made of coconut oil are more likely to irritate the skin than soaps made from tallow or olive oil.

A large internationally known manufacturer of soap was about to

This paper is the result of patch tests on 200 humans comparing the irritant action of a new deodorant soap with a toilet soap base and with a deodorant soap on sale for many years. This investigation was sponsored by Armour Toiletries, Chicago, Illinois.

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place on the market a new deodorant soap. It contains Hexachlorophene (bis-3,5,6-trichloro-2hydroxy phenyl methane), an antiseptic and germicide which, when added to soap, has been proven by experiments to rid the skin of far more germs than can the soap itself.[3,10,11,12] It has also been shown that repeated washing with a soap containing Hexachlorophene will cause an accumulation of this chemical on the skin and thus prevent the multiplication of the bacteria which may not have been destroyed or removed.[2,9] Since the offensive odor of the perspiration has been shown to be mainly due to bacterial decomposition of the naturally odorless skin excretions (perspiration, sebum) which are deposited and accumulate on the skin,[4,5] especially in the axillae, the groins, under the breasts, and other skin folds, it was thought the continued use of a soap containing Hexachlorophene, which so markedly reduces the bacterial flora of the skin, would prevent the decomposition of the skin excretions and hence act as a deodorant soap. Unpublished experiments performed by a well-known consulting laboratory have actually proved this to be the case.[1]

Before placing the new deodorant soap on public sale, the manufacturers, wishing to safeguard against dermatitis, compared its action on the skin with soaps which have long been on the market without causing any undue amount of dermatitis, and this paper records the experiments devised to make this comparison.

In 1940, the author published a method for testing the possible skin-irritant properties of new substances before placing them on sale.[8]

This method consists of first performing two series of patch tests, about ten days apart, on at least 200 humans with the new substance, and using as a control patch a similar substance that has long been in public use without causing any undue amount of dermatitis. (This recognizes the fact that there is scarcely any substance to which someone may not be sensitive or become sensitized).

A comparison of the positive reactions obtained from the test product with the positive reactions obtained from the product long on the market without causing any undue trouble is made. If there are no more reactions from the test product than there are from the product long on the market, then the test product can be placed on trial sale in a small community, 5,000 to 10,000 people, for a month or more for the purpose of finding the skin-irritant potential. The results of this test will determine whether the product can be placed on the general market without endangering the public.

The above-described method was devised, having in mind fabrics for wearing apparel in which the concentration of the chemicals is constant and evenly divided throughout the fabric.

It is apparent that when liquids, oils, fats or powders are to be tested, comparative patch tests, in order to be of value, must be performed with equal amounts of the substances to be compared, placed on equal areas of skin, and on similar places on the body. (The reactivity of the skin on

different parts of the body may vary because of normal anatomical and physiological variations).

Such a method of comparative patch testing can easily be performed by the standardized patch test devised by the author.[6]

With this method of patch testing, definite amounts of substances can be applied to constant or definite and similar areas of skin, for definite periods, thus enabling a comparison of the skin-irritant properties of several substances. The effect on the skin of various concentrations of the same substance can also be determined.

The standard Schwartz patch consists of a square piece of flannel 3 sq. cm. in area, to which 0.2 c.c. of a liquid is applied with a graduate pipette, or hypodermic syringe or an eye dropper. The moistened flannel is placed on the skin, covered with a piece of elastic fabric of a special pattern, in the center of which there is an insulating square piece of uncoated cellophane (regenerated cellulose) measuring 1½ inches on each side. The cellophane is placed directly over the moist flannel. When patching with greases, definite amounts of the greases are spread over the flannels. The same applies to powders.

Thresholds of sensitivity can be determined by the application of ascending amounts of the liquid, grease or powder. The time factor can also be controlled.

The above-described method of patch testing was used to compare the effect on the skin of a new deodorant soap, with the effect on the skin of a base toilet soap and with a deodorant soap which has been on the market for many years.

In 1934 Schwartz[7] prepared a special soap made from sodium hydroxide and first pressing of olive oil, and found that a 3 per cent solution placed on the skin for twenty-four hours, as a patch test, would in most cases cause only a slight branny desquamation. Therefore, it was decided to apply as the first or sensitizing patch a 3 per cent solution of each of the three soaps.

The compositions and pH of the applied soap solutions were as follows:

Base soap consisted of 80 per cent of a sodium hydroxide, tallow soap plus 20 per cent of a sodium hydroxide, coconut oil soap; pH 10.3.

New deodorant soap consisted of the above base soap to which was added 2 per cent Hexachlorophene and 1 per cent perfume; pH 10.2.

Control deodorant soap was a soda tallow-coconut soap, plus a chemical deodorant; pH 10.2.

The results of the patch tests would show the effect on the skin of the action of the additives to the base soap and also give a comparison of patch tests with the test soap, with patch tests with the same concentrations of the well-known control deodorant soap (which has been sold for many years without undue harmful effects).

Two hundred white subjects were used. These were about two-thirds male and one-third female.

The following technique was used: 0.2 c.c. of a 3 per cent solution of soap was applied to a piece of absorbent flannel 3 sq. cm. in area. This was sufficient to wet the flannel without having the solution drip out of it. The wet flannel was applied to the skin of the upper arms, forearms, back, or thighs as desired by the subjects. However, all three patches were applied to similar locations on the same subject. The flannel was sealed on the skin with a piece of elastic fabric to which adhered a piece of uncoated cellophane 1½ in. sq. which covered the wet flannel. The patches were removed at the end of twenty-four hours and the reactions noted.

193 subjects returned for reading of reactions.

Degree of Reaction	++	+	?	—
Base Soap	0	180	0	13
New Deodorant Soap	0	182	0	11
Control Deodorant Soap	2	184	0	7

This first test showed that a 3 per cent solution of these soaps permitted to remain on the skin for twenty-four hours under a sealed patch will cause reactions on nearly all subjects, i.e., is a primary skin irritant.

In order to determine what concentration of the soaps was to be placed on the skin in the second series of tests (to determine sensitization), six of the subjects who had undergone the first series of tests, and reacted to all three patches of 3 per cent strength, were patched with 1 per cent and 2 per cent solutions of the base soap and of the control deodorant soap using the technique described above. The patches were removed at the end of twenty-four hours. All the subjects showed reaction to the 2 per cent solutions. Only three subjects showed reactions to the 1 per cent solution. Therefore it was decided that 1 per cent solutions of the three soaps were to be used in the second series of patch tests to determine the number sensitized by the first series.

The second series of patch tests were applied fifteen days after the first series. The same technique was used as in the first series.

193 subjects were patched with 1 per cent solutions of the three soaps according to the technique described above. The pHs of these solutions were: Base Soap—9.9; New Deodorant Soap—9.9; Control Deodorant Soap—9.9.

The patches were removed at the end of twenty-four hours and the reactions were read as follows:

Degree of Reaction	++	+	?	—
Base Soap	5	102	36	50
New Deodorant Soap	0	51	38	104
Control Deodorant Soap	6	114	32	41

Twenty-four hours after the removal of the patches, the subjects were again examined for delayed reactions. At this examination the following gradations of the reactions were made.

Results of reaction readings twenty-four hours after removal of second series of patches:

Degree of Reaction	++	+	?	—
Base Soap	1	89	26	65
New Deodorant Soap	0	33	25	123
Control Deodorant Soap	4	95	24	58

It will be noted that there were fewer reactions twenty-four hours after the removal of the patches, than there were immediately upon their removal. This shows that the reactions which had disappeared twenty-four hours after the removal of the patches were definitely reactions of primary irritation.

The results of these tests showed that:

1. A 3 per cent solution of all three soaps remaining on the skin for twenty-four hours in the form of a standard covered patch test will cause reactions on more than 90 per cent of subjects.
2. A 1 per cent solution of the base soap under the same condition caused definite reactions on about 46 per cent (taking the final reading twenty-four hours after removal of one per cent solution patch).
3. A 1 per cent solution of the same base soap to which 2 per cent Hexachlorophene and 1 per cent perfume was added, caused definite reactions in only about 17 per cent under the same conditions.
4. A 1 per cent solution of the control deodorant soap caused definite reactions in about 51 per cent.
5. None of the subjects who showed no reactions to the first series of patches of the 3 per cent solutions showed reactions to the second series of patches of 1 per cent solutions.
6. There was no significant difference between the percentage of reactions to the base soap and to the control deodorant soap.

DISCUSSION

There were fewer reactions to the second series of patches than to the first series. This is unusual, but may be because all the subjects were soap users, and whatever sensitizations they could acquire to soap they had already acquired before the tests began. Or it may be that the sensitization which may have been acquired by reason of the application of the 3 per cent solution patches was more than counterbalanced by the fact that the second series of patches were only one-third the strength of the first series.

In the second series of patches there were less reactions to the new deodorant soap than to the base soap from which it was compounded despite the fact that in addition to the base soap the new deodorant soap contained 2 per cent of Hexachlorophene and a perfume. It may be possible that the sensitizing properties of 1:5,000 of Hexachlorophene (the amount contained in the second series of patches) would not show, where-

as the superfatting of the soap by the presence of the essential oils of the perfume and by the Hexachlorophene may have reduced the irritant properties of the base soap.

CONCLUSIONS

1. The above described standardized method of patch testing can be used satisfactorily to compare the relative skin-irritant properties of various soaps.*

2. Although the closed patch tests, as described, show a considerable number of reactions among the test subjects, all of them use soap for the cleansing of the skin without any skin irritation. This shows that the closed patch test with soap is more irritant than actual usage of soap.

3. The base soap and the control deodorant soap, although showing more reactions under the above-described patch test conditions than the new deodorant soap, have been actually used by the public in large quantities for many years without any undue number of cases of sensitization.

4. The new deodorant soap showing considerably less reaction under the above described patch test conditions, should cause even fewer sensitizations when used by the public than do the base soap and the control deodorant soap (which has been on the market for many years).

5. Since this experiment was conducted, several million cakes of the new deodorant soap have been sold with no proven cases of sensitization having been reported from its use.

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*The relative primary irritant and sensitizing properties of other chemicals can also be ascertained by this method.

THE VARIABILITY OF ORAL ANTIHISTAMINIC THERAPY

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IN the course of investigating the ability of various antihistaminic agents to prevent the decrease in vital capacity induced in some asthmatic patients by histamine, we have encountered three individuals who showed no such protective action following the oral administration of diphenhydramine or tripelennamine. In each case, the protecting capacity of the drug after parenteral administration was similar to that manifested by other patients, in whom no demonstrable impairment of antihistaminic activity was observed following their oral ingestion.

The technique of these studies has already been reported in detail.[2,3,6] Essentially, it consists of observing the effect of a therapeutic or protecting agent in modifying or preventing the decrease in vital capacity induced by the administration of any bronchospastic agent, in this case histamine diphosphate, given intravenously or as an aerosol. Injections or aerosolizations of the bronchospastic agent are repeated until the effect of the protecting agent has disappeared, when the decrease in vital capacity returns to the pre-protection levels. The degree of protection present at any time has been described in terms of the following equation:

$$P = \frac{C - E}{C \times 100,}$$

where P = degree of protection in per cent, C = control decreases in vital capacity, measured prior to administration of the protecting agent, and E = the decrease observed when histamine is administered at any interval after the protecting agent has been given. We have considered protection values below 40 per cent of doubtful significance, in view of the many sources of error inherent in any technique of clinical assay. Both diphenhydramine and tripelennamine were administered orally and rectally in 50 mg. doses; the intravenous dose of tripelennamine hydrochloride was 25 mg, of diphenhydramine hydrochloride, 28 mg. All aerosols were produced in the Vaponefrin nebulizer. Six inhalations were

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given as a dose. A 2 per cent tripelennamine solution and a 1.4 per cent diphenhydramine solution were used for the aerosols.

Histories of the three individuals who did not exhibit protection following the oral administration of diphenhydramine or tripelennamine are presented below.

Case 1 (G. Bro.).—This man, aged thirty-three, married, white, a street-car operator, was seen at the height of the ragweed season (1948) with moderate severe asthma and hay fever. His asthma was perennial, but was always worse in August and September. No previous or family history of allergy could be elicited. Positive intradermal skin tests were elicited to dust, to mixed feathers, and to dog and cat hair. In spite of large amounts of seven different oral antihistamines, no relief from his distressing hay fever and bronchospasm was obtained, even though drowsiness was frequently produced. The bronchospasm was relieved on a regimen of bronchodilator aerosols as needed and rectal aminophyllin two to three times daily, but the hay fever was so distressing as almost to incapacitate him.

On two occasions the oral administration of 50 mg of tripelennamine resulted in no modification of the vital capacity response to subsequently administered histamine aerosols. When the same dose of tripelennamine was administered rectally, 40 per cent protection against histamine aerosol was apparent within fifteen minutes, and protection became complete (100 per cent) sixty minutes after tripelennamine had been administered rectally. Complete protection lasted for about sixty minutes and the degree of protection then decreased gradually, falling to 40 per cent again 165 minutes after tripelennamine had been administered and disappearing completely 240 minutes after rectal tripelennamine. Aerosol tripelennamine even in the minute dose administered (six inhalations of an aerosol made from a 2 per cent solution of tripelennamine hydrochloride) yielded complete (100 per cent) protection against the decrease in vital capacity induced by subsequently administered histamine aerosol. This protection was apparent immediately and had already begun to decrease by the end of one-half hour. Significant (40 per cent) protection lasted for approximately 160 minutes.

In the same individual no protection against the effects of histamine aerosol was apparent after the oral administration of 50 mg of diphenhydramine. When the same dose was administered rectally, 47 per cent protection was apparent one-half hour later. This level was maintained for ninety additional minutes; the protection disappeared by the end of the third hour. When 28 mg of diphenhydramine was administered intravenously, 82 per cent protection was apparent five minutes later. This level was then maintained for 180 minutes, following which the degree of protection rapidly diminished. Protection was no longer detectable four hours after intravenous diphenhydramine.

In this individual it is apparent that either diphenhydramine or tripelennamine administered rectally is able to prevent the effects of histamine aerosol, but both are totally ineffective when given by mouth. Aerosol tripelennamine and intravenous diphenhydramine are likewise effective.

Striking clinical relief of both asthma and hay fever followed the addition of 2 per cent tripelennamine aerosol and of rectal tripelennamine (50 mg two to three times daily) to the above regimen.

Case 2 (A. Bai.).—This housewife, aged thirty-four, was seen in September, 1948. She gave a history of perennial bronchial asthma of twenty-seven years' duration, requiring five hospitalizations. For the past two ragweed seasons, she had experi-

enced moderate hay fever, which disappeared with the first frost, and also mild atopic dermatitis of the face and left forearm. Significantly positive intradermal skin tests to dust and to mixed feathers were elicited. She had obtained no benefit from tripelennamine, 50 mg orally, three times daily for two weeks, although this preparation produced distressing dizziness and drowsiness. Bronchodilator inhalations were effective in terminating a paroxysm, and some prophylactic relief was afforded by aminophyllin suppositories. Diphenhydramine, when given orally in moderately large doses, did not affect her hay fever or bronchospasm appreciably, even though drowsiness was produced.

After the oral administration of 50 mg of tripelennamine hydrochloride, no alteration in the response to histamine aerosol could be detected. When 25 mg was administered rectally, however, significant (40 per cent) protection against histamine aerosol was apparent after forty-five minutes. A maximum protective level of 83 per cent appeared at the end of 120 minutes, following which the degree of protection rapidly diminished. When 25 mg of tripelennamine hydrochloride was administered intravenously, complete protection was immediately attained. One hundred per cent protection persisted for sixty minutes, following which the degree of protection gradually lessened, falling below 40 per cent 120 minutes after the administration of tripelennamine. Aerosol tripelennamine was likewise potent, a peak level of 83 per cent being attained immediately after its administration. Significant (40 per cent) protection persisted for 155 minutes after the administration of aerosol tripelennamine.

Rectal and aerosol tripelennamine again yielded clinical relief.

Case 3 (J. Ste.).—This man, aged sixty-three, white, retired, was seen in April, 1948, and gave a three-year history of perennial asthma. Prior to being followed by the clinic, he had been digitalized for what was considered cardiac decompensation. He has been comfortably maintained on rectal aminophyllin, and obtained prompt relief of his acute paroxysms with bronchodilator aerosols. He has observed that whereas a combination of diphenhydramine and aminophyllin (Hydryllin), taken orally, afforded some relief of his nocturnal asthmatic distress, large doses of tripelennamine alone produced neither drowsiness nor similar nocturnal relief.

After the oral administration of 50 mg of tripelennamine, protection against the effects of intravenous histamine was entirely lacking (two trials). When the same dose of tripelennamine was administered rectally, 67 per cent protection was apparent after thirty minutes. A peak level of 83 per cent protection was attained at 90 minutes. Significant protection persisted for an additional 150 minutes, following which the degree of protection rapidly diminished. As in previous cases, intravenous and aerosol tripelennamine were also effective in preventing the drop in vital capacity induced by histamine. Twenty-five mg of tripelennamine administered by vein gave immediate complete protection against the effects of intravenous histamine. The degree of protection then gradually diminished but was still significant at the end of 230 minutes. Immediately after aerosol tripelennamine 96 per cent protection was observed. Significant protection was still present 140 minutes later, at the conclusion of this particular experiment.

In this individual, although oral tripelennamine was ineffective, oral diphenhydramine yielded significant protecting levels. A peak of 53 per cent protection was observed 90 minutes after the administration of 50 mg of diphenhydramine by mouth. Significant protection was maintained until 210 minutes after diphenhydramine, at which time the experiment was concluded. As was the case with tripelennamine, diphenhydramine was also active when given by other routes. When 50 mg was administered rectally, a maximum protective level of 80 per cent was attained sixty

TABLE I. THE ANTIHISTAMINIC PROTECTIVE CAPACITY OF DIPHENHYDRAMINE AND TRIPELENNAMINE IN THREE ASTHMATIC SUBJECTS WHO SHOW ABSENT PROTECTION AFTER ORAL ADMINISTRATION OF THESE AGENTS

Case	Route of Histamine	Antihistaminic Agent and Route	Peak Level of Protection (%)	Duration of Significant (40%) Protection Minutes
G. Bro.	Aerosol	Tripelennamine, oral	0	0
		rectal	100	150
		aerosol	100	160
		Diphenhydramine, oral	0	0
		rectal	47	110
		intravenous	82	210
A. Bai.	Aerosol	Tripelennamine, oral	0	0
		rectal	83	95
		aerosol	83	155
		intravenous	100	120
J. Ste.	Intravenous	Tripelennamine, oral	0	0
		rectal	83	255
		aerosol	96	140
		intravenous	100	230
		Diphenhydramine, oral	53	120
		rectal	80	195
		aerosol	77	70
		intravenous	80	180

minutes later. Significant protection persisted for 150 minutes longer, the duration of the experiment. After aerosol diphenhydramine, there was 77 per cent immediate protection against the effects of intravenous histamine; significant protection lasted seventy minutes. When 28 mg of diphenhydramine hydrochloride was given by vein, 80 per cent immediate protection was observed against the effects of intravenous histamine. The degree of protection gradually diminished; significant protection persisted for 180 minutes.

These three individuals were subjected to protection studies because of clinical indications that orally administered diphenhydramine and tripelennamine were ineffective in controlling their symptoms. For comparison we have performed similar studies on four other individuals in whom these agents exhibit the usual dramatic clinical effect. In each of these, both diphenhydramine and tripelennamine exhibited significant protection when given orally. In approximately one half of this latter group of studies, rectal administration of diphenhydramine or tripelennamine again yielded more intense and prolonged protection than followed oral administration.

The pertinent data with regard to our three cases showing absent protection after oral administration of diphenhydramine and tripelennamine are summarized in Table I. Similar data with regard to the other four patients who exhibit the anticipated protection levels are summarized in Table II.

DISCUSSION

Oral administration of antihistaminic agents in some asthmatic patients fails to produce significant protection in the laboratory against histamine-induced dyspnea and bronchospasm, which parenteral administration con-

TABLE II. THE ANTIHISTAMINIC PROTECTIVE CAPACITY OF DIPHENHYDRAMINE AND TRIPELENNAMINE IN FOUR ASTHMATIC SUBJECTS WHO SHOW HIGH PROTECTION AFTER ORAL ADMINISTRATION OF THESE AGENTS

Case	Route of Histamine	Antihistaminic Agent and Route		Peak Level of Protection (%)	Duration of Significant (40%) Protection, Minutes
A. Nig.	Intravenous	Tripelennamine,	oral	80	150
			rectal	100	210
			aerosol	60	30
			intravenous	100	300
		Diphenhydramine,	oral	100	220
			rectal	100	285
			aerosol	0	0
			intravenous	100	210
M. Ger.	Intravenous	Tripelennamine,	oral	80	200
			rectal	100	260
			aerosol	70	110
			intravenous	100	240
		Diphenhydramine,	oral	74	200
			rectal	100	260
			aerosol	100	40
			intravenous	100	230
	Aerosol		oral	100	210
			rectal	100	195
			aerosol	100	75
			intravenous	70	180
W. Hun.	Aerosol	Tripelennamine,	oral	70	230
			rectal	100	170
			aerosol	88	60
			intravenous	100	285
		Diphenhydramine,	oral	100	170
			rectal	100	175
			aerosol	100	100
			intravenous	100	310
P. Del.	Intravenous	Tripelennamine,	oral	46	20
			rectal	72	210
			aerosol	82	150
			intravenous	85	150
		Diphenhydramine,	oral	65	170
			rectal	90	255
			aerosol	70	40
			intravenous	100	240

sistently affords. In other individuals, oral administration yields protective capacities similar to those found after intravenous or rectal administration.

At the present time, we can only speculate upon the cause of this phenomenon. One might be dealing with lack of gastric or duodenal absorption, with enzymatic digestion, or with hepatic inactivation of these agents. The last possibility seems most probable, since the only common factor in the other, efficient routes of administration, is the by-passing of the portal circulation. McGavack,[4] Arbesman[1] and Serafini[7] have also commented on the variability of "absorption rates" in man of antihistaminic drugs, the former employing blood level determinations, and the latter using histamine and antigen-antibody skin reactions.

We agree with McGavack[5] that there is an apparent dissociation between the antihistaminic effects and the side reactions of these compounds. This was particularly exemplified by the occurrence of drowsiness in Cases 1 and 2, following the ingestion of diphenhydramine or tripelennamine, at a time when no protection was being afforded.

SUMMARY

In three asthmatic patients, orally administered tripelennamine and/or diphenhydramine were ineffective in preventing histamine-induced dyspnea and bronchospasm. In these patients, the same drugs were quite effective after rectal, intravenous, or aerosol administration. The mechanism of this phenomenon is unknown, but it may represent hepatic inactivation. The sedative effect of these agents may persist in the absence of any appreciable antihistaminic effect.

These observations in the laboratory suggest the employment of rectal, aerosol and intravenous antihistaminic agents in the clinical management of patients with hay fever and bronchial asthma who do not respond to these agents when administered orally.

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ALLERGY SECTION AT MINNESOTA MEDICAL MEETING

The American College of Allergists, by invitation from the Minnesota State Medical Association, conducted a half-day special session June 13 at Duluth, Minnesota. An introductory talk was given by Fred W. Wittich, M.D., followed by a paper entitled "Skin Allergy, Newer Trends in Diagnosis and Management" by Stephan Epstein, Marshfield Clinic, Marshfield, Wisconsin, and Clinical Associate, Professor of Dermatology, University of Minnesota. "Respiratory Allergy; Hay Fever—Including Nonspecific and Specific Therapy" was presented by George Loomis, M.D., Winona, and Fred W. Wittich, M.D., Minneapolis. "Allergic Rhinitis and Bronchial Asthma" was prepared by Albert V. Stoesser and Lloyd S. Nelson of the University of Minnesota. The concluding paper was "Status Asthmaticus" by William S. Eisenstadt of Minneapolis.

THE PRECIPITATION OF REAGIN AND THERMOSTABILE (BLOCKING) ANTIBODY WITH AMMONIUM SULPHATE IN RAGWEED-SENSITIVE SERUM

I. Technique

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COOKE[3] *et al* demonstrated the production of another antibody in the sera of ragweed-sensitive patients after the injection of ragweed antigen. Loveless[7] further elaborated on its inhibiting or blocking character and its thermostability compared to reagin. Frank and Gelfand[4] enlarged on its specificity and non-existence in other immune sera. Hampton[5] *et al*, by using a ragweed-antiragweed rabbit serum system, were able to obtain increased precipitation due to human thermostabile antibody.

The general concept of blocking antibody is well accepted. Weiner[9] defines the immune state as one in which a large amount of blocking antibody, due to the introduction of antigen, is present in the body fluids. On the opposite side, Bronfenbrenner[1] questions that reagin and blocking antibody are two distinct antibodies. Among others, he cites the work of Kleczkowski[6] to support this view. The latter, using tobacco mosaic virus and tomato bushy stunt virus as antigens and the agglutination test, attempts to show that the heating of the antibody alone did not produce blocking antibodies, but that the heating of the euglobulin antibody containing fraction in the presence of other proteins such as albumin, or as in whole serum, produces a protein complex which is in effect the blocking antibody. The author accepted the challenge of determining whether Kleczkowski's illuminating work supporting the unitarian theory could be duplicated on the sera of untreated and treated ragweed-sensitive patients.

This first paper deals primarily with the techniques used to obtain suitable euglobulin and other antibody containing fractions.

MATERIALS

The serum from a ragweed-sensitive patient who had received ragweed extract injections previously but not for the past six years, and whose serum contained both reagin and thermostabile antibody, was the source from which all the preliminary fractions were precipitated. Pooled sera from non-treated ragweed-sensitive patients was the reservoir of reagin for thermostabile antibody titrations. Saturated, two-thirds- and one-third-saturated ammonium sulphate solutions were used for precipitating and washing the various fractions. Mixed ragweed extract, measured in protein nitrogen units, was used for testing. Merthiolate 1-7,500 was the preservative in some sera. A Swinney Seitz filter adapter offered a convenient means for Seitz filtration in others.

Presented before the Sixth Annual Meeting of the American College of Allergists, St. Louis, Missouri, January 15-18, 1950.

METHOD

The essential method tried is that of Kleczkowski, who precipitated euglobulin by one-third saturation of the serum with ammonium sulphate, filtering the precipitate, washing the same with one-third-saturated ammonium sulphate, dissolving the precipitate on the filter paper in water, and dialyzing the solution. The insoluble euglobulin in the dialysis bag after dialysis was filtered off, and only the soluble globulin in the filtrate was used for agglutination tests. Attempts to duplicate this technique exactly in ragweed-sensitive serum encountered difficulties in obtaining an antibody-containing fraction. Several variations of the method were tried. Essentially, they consisted of trials with a protein fraction at either a 28 per cent, 33 per cent, 43 per cent or 50 per cent ammonium sulphate saturation of the serum. Ammonium sulphate in proper strength and amount was added to 1 to 5 cc aliquots of serum, directly (whether slowly or rapidly did not seem to influence the final antibody content). The precipitates were at first filtered but later centrifuged at 4,500 RPM for thirty minutes. The filtration method required large quantities of diluent to insure dissolving the precipitate on the filter paper. These dilute solutions after dialysis were devoid of antibodies. Dialysis was carried out at first against water alone. Later saline was tried, but was replaced by running tap water for two to twelve hours followed by saline. As in the work of McKhann and Chu[8] it was found that water dialysis alone gave large quantities of insoluble euglobulin in the dialysis bag and a pH about 4; and frequently antibodies could not be detected. Originally, merthiolate was added for preservative. Later the end product was Seitz filtered.

RESULTS

The results obtained may be briefly stated, and are readily visible in the accompanying table. The percentage yields were not constant for the various fractions, evidenced by the variability (within limits) of the protein percentages obtained. The water dialysis tended to give a lower yield than water plus saline dialysis. Where reagin was present in a fraction it existed quantitatively, when tested, in approximately the same amount, regardless of the method of preparation of the fraction. Whenever reagin was present, if thermostabile antibody was tested for, it was present, and again quantitatively, in constant amounts, regardless of the nature of preparation of the fraction. Dilution of redissolved precipitate before dialysis seemed to result in loss of antibody content; conversely, dilution after dialysis did not affect antibody content.

DISCUSSION

Kleczkowski states, "antibodies . . . heated with euglobulin fractions of the antisera only . . . can still unite with and flocculate their antigens

METHOD OF PREPARATION, PROTEIN YIELD, TEST FOR ANTIBODIES (REAGIN AND THERMOSTABILE) IN RAGWEED SENSITIVE SERUM PRECIPITATED WITH $(N_4H)_2SO_4$

Sample	$(NH_4)_2SO_4$ Saturation	Filtered	Centrifuged	Dialysis			Protein Insoluble Euglobulin	Grams/100 cc		Antibodies		Merthiolate	Seitz Filtered
				H_2O	Saline	H_2O plus Saline		Soluble Globulin	Dilution for Testing	Reagin Present Titre	Thermostabile Titre		
F1	33%	X	—	X	—	—	.375	—	—	No	—	X	—
F20a*	33%	X	—	X	—	—	—	1.13	1–10	none	none	—	X
F20b*	33%	X	—	—	X	—	—	1.38	1–10	none	none	—	X
F21*	33%	X	—	—	—	X2**	.19	.61	1–10	none	none	—	X
F22*	33%	—	X	—	—	X2**	—	—	1–5	none	none	—	X
F3	33%	—	X	X	—	—	.49	.31	—	—	—	—	—
F4	33%	—	X	X	—	—*	.88	1.25	—	—	—	—	—
F5	33%	—	X	X	—	—	.425	.5	—	—	—	—	—
F11	33%	—	X	—	—	X12**	—	—	1–1	none	none	X	—
F12	50%	—	X	—	—	X12**	—	—	1–1	none	none	X	—
F17	33%	—	X	—	—	X7**	—	—	1–1	600PNU	400PNU	—	X
F18a	33%	—	X	—	—	X3**	—	1.4	5:4	600PNU	400PNU	—	X
F19a	33%	—	X	—	—	X9**	—	1.72	1:1	600PNU	400PNU	—	X
F8a	28%	—	X		Not Dialyzed		—	—	1:1	none	—	X	—*
F8b	50%	—	X		Not Dialyzed		—	—	1:1	yes	—	X	—
F8c	50%	—	X	X	—	—	—	—	1:1	yes	—	X	—
F9a	50%	—	X		Not Dialyzed		—	—	1:10	yes	—	X	—
F9b	43%	—	X		Not Dialyzed		—	—	1:10	yes	—	X	—
F9a	50%	—	X	—	—	X12**	—	—	1:10	yes	—	X	—
F9b	43%	—	X	—	—	X12**	—	—	1:10	yes	—	X	—
F10abc	50%	—	X		Not Dialyzed		—	—	1:2	yes	—	X	—
F10ab	50%	—	X	—	—	X7**	—	—	1:2	yes	—	X	—
F15	50%	—	X	—	—	X3**	—	—	1:2	yes	—	—	X
F16	50%	—	X	—	—	X2**	—	—	1:1	600PNU	400PNU	—	X
F18b	33–50%†	—	X	—	—	X3**	—	.956	5:4	600PNU	—	—	X
F18c	50%	—	X	—	—	X3**	—	2.90	5:6	600PNU	400PNU	—	X
F19b	33–50%†	—	X	—	—	X9**	—	1.55	1:1	600PNU	400PNU	—	X
F19c	50%	—	X	—	—	X9**	—	2.24	1:1	600PNU	400PNU	—	X

*In these samples, the precipitate after ammonium sulphate saturation was redissolved and diluted before dialysis. All other precipitates were redissolved in an amount of saline to equal the original volume or close to it. Any dilutions for testing were made, subsequently, after dialysis.
**The number after the X indicates the number of hours dialyzed against running tap water, followed by saline
†Fraction between 33 percent and 50 percent saturation, after 33 percent fraction has been salted out.

. . . antibodies . . . heated in presence of protein fractions other than euglobulin . . . can combine with their antigen but cannot cause flocculation . . . and can subsequently prevent the latter from being precipitated by unchanged antibody." However, as he states in his article, he filtered off the insoluble euglobulin after dialysis, and used only the soluble globulins for his tests. Cohn[2] *et al,* used Tisellius' electrophoretic technique to check the globulins precipitated by various saturations of ammonium sulphate. They found that one-third saturation resulted in a precipitate, of which only about one third was euglobulin (insoluble in water), and that the rest was chiefly water soluble gamma globulin; that at 40 per cent saturation, alpha, beta and gamma globulins were present plus about one third insoluble euglobulin; whereas, at 50 per cent saturation, alpha and beta globulins were present chiefly, with about 6 per cent of insoluble euglobulins.

Cohn's observations relate to the present problem in two ways: (1) Kleczkowski, while talking about insoluble and soluble euglobulin, actually discarded the former and used a gamma globulin for his tests; (2) the results in the present paper demonstrate the presence of reagins and thermostabile antibodies in the 33 per cent ammonium sulphate saturation fraction, composed chiefly of euglobulin and gamma globulin, and also in the 50 per cent fraction composed chiefly of alpha and beta globulins. Although Kleczkowski used in reality gamma soluble globulin, his theory of the effect of heating antibodies may still be valid. It will be discussed in a subsequent paper which gives results relating to that aspect of the problem.

McKhann and Chu reduced dialysis against running tap water to twelve hours, in order to reduce the insoluble euglobulin thrown out of solution. In the present instance it was impossible to keep all the euglobulin in solution after dialysis, although the amount precipitated out could be reduced markedly, by cutting water dialysis to two hours. However, subsequent tests revealed that the quantitative presence of reagin and thermostabile antibody seemed to be independent of the presence of euglobulin (unless that small amount which remained in solution determined the antibody activity). Seitz filtration removed all insoluble euglobulin, without affecting antibody titre.

SUMMARY

1. Human serum known to contain ragweed reagins and the thermostabile antibody was saturated with one-third and 50 per cent ammonium sulphate. The resultant precipitate was either filtered and redissolved or centrifuged and redissolved in saline. It was then dialyzed against water or saline, or both. The insoluble euglobulin precipitate which formed on dialysis was filtered off. The soluble globulins which remained were tested for reagins and thermostabile antibodies.

2. Dealing with small quantities of serum, various difficulties were encountered in attempting to obtain fractions of constant protein content. Despite the variations in total protein content from different samples and different fractions, the antibody content of all was constant.

3. The one-third ammonium-sulphate-saturation fraction is composed chiefly of soluble gamma globulin after dialysis; the 50 per cent fraction is composed chiefly of alpha and beta globulins. Both fractions showed the presence of both reagins and thermostabile blocking antibodies in quantitatively about the same amounts.

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TRIMETON AND CHLOR-TRIMETON MALEATE

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INGESTION OF 1250 MG OF DEMEROL (ISONIPECAINE) WITH SUICIDAL INTENT

A Case Report

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A CASE of Demerol poisoning is reported in which 1250 mg in a single dose was taken with suicidal intent. It is of interest that this quantity of the drug produced relatively mild toxic symptoms and there was a rapid recovery with no apparent sequela.

CASE REPORT

The patient, a white man, aged thirty-four, entered the Norton Memorial Hospital at 8:30 a.m., August 20, 1949, complaining of drowsiness, sweating, dryness of the mouth and nausea following the ingestion of twenty-five 50 mg Demerol tablets. He had vomited about thirty minutes after taking the drug and repeatedly thereafter.

The patient had domestic difficulties which caused him to become profoundly frustrated and depressed. At 3 a.m. in the presence of a witness, he took twenty-five 50 mg Demerol tablets.

This patient had been under my care since 1942 and had suffered from asthma and saccular bronchiectasis since 1940. In 1942 a bronchogram was made which showed bilateral bronchiectasis involving all the lobes of the lungs. The patient moved to Denver, Colorado, where he stayed from 1942 to 1945; but there was little, if any, improvement in his condition. Another bronchoscopy and bronchogram was made July 8, 1949, which showed cylindrical and saccular bronchiectasis involving all lobes bilaterally. The bronchiectasis was so extensive that it was advised that no benefit could be offered by surgery.

Aside from bronchiectasis and chronic maxillary sinusitis, the patient showed no other significant disability. Routine allergy tests were negative. Sputum examinations showed no tubercle bacilli. Sputum cultures showed Gram positive short chain streptococci. The Kahn was negative and the urine and blood counts were normal.

Treatment was entirely symptomatic. Intramuscular and aerosol penicillin, as well as sulfa drugs, were used from time to time with varying results.

Oral Demerol was used occasionally at night. The usual expectorants such as iodides, ammonium chloride, together with postural drainage, were used with fair degree of regularity. Since November, 1948, Isuprel aerosol was used with some relief of the asthma and since June 14, 1949, 7.7 grs. of sulfadiazine had been given daily. On June 22, 1949, the patient was seen at the office. He had gained weight and was feeling well. On August 18, a prescription was requested for twenty-five Demerol tablets; and, since the patient lived out of the city, this amount was sent to him on prescription from a local druggist.

On physical examination upon admission to the hospital, the patient seemed drowsy and confused. He perspired freely and complained of extreme dryness of his mouth. The pupils were equal and regular and responded to light. The heart appeared normal and his breathing remarkably clear. The temperature was 99° F, pulse 100, blood pressure 108/60. The blood count showed 13,580 WBC, of which

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90 per cent were neutrophiles. The urine was normal except for three to four pus cells and occasional granular cast per HPF.

On admission a gastric lavage was done and 20 mg of Benzedrine was given intramuscularly. Blood pressure determinations were made at fifteen-minute intervals for four hours, but there was remarkably little variation from the admission recording.

The following day the blood pressure was found to be 118/70. He complained of cramping pains of both thighs, and the asthmatic condition was worse than for some time. Ammonium chloride and potassium iodide were given by mouth together with aminophyllin suppositories.

He was seen by a psychiatrist, who felt that the patient was a constitutionally inadequate individual. Note was made that the patient's mother was dead and that he had in recent years developed considerable dependency upon his mother-in-law. The psychiatrist stated that since the emotional problem had been solved, no further attempt at self-destruction was anticipated. The patient was given Benzebar tablets t.i.d. in addition to the medications for his pulmonary condition. He was discharged from the hospital August 23, 1949.

DISCUSSION

The above case suggests that Demerol has a relative-low toxicity and that it is a fairly safe drug to prescribe to patients who might deliberately or otherwise take more than a therapeutic dose. The fact that the patient vomited within an hour following the ingestion of the Demerol and vomited repeatedly thereafter until admission to the hospital of course may have prevented the absorption of the entire amount of the drug.

The United States Dispensatory (Osal-Farrar, 24th ed., page 1425) states "Administration of Demerol hydrochloride to man in doses of 50 to 150 mg at three- or four-hour intervals, as it is given clinically, produces mild circulatory or respiratory effects only occasionally. Prolonged use has resulted in no alternation of the hematopoietic system or impairment of kidney functions. Blood sugar levels are not altered. In bed patients receiving the drug by the parenteral route, dizziness is observed in approximately 22 per cent, nausea and vomiting in 4 and 8 per cent, respectively, of cases; in ambulatory patients these effects are both more frequent and more severe. Respiration and dryness of the mouth may at times be marked. Since these reactions subside if the drug is continued they are not an indication to discontinue medication. Excessive doses, as employed in abuse of the drug, may result in tremors and possibly convulsions; the latter have occurred if the dose exceeds 0.2 gm every two hours. With therapeutic doses cerebral irritation, such as tremors and unco-ordinated muscular movements, may occur in an occasional patient. . . . Demerol hydrochloride should be administered with caution, if at all, to patients with intracranial lesions."

Andrews[1] administered parenterally an initial dose of 100 mg of Demerol hydrochloride, and thereafter, for ten consecutive weeks, doses

of a size and frequency chosen by the men, with limits of 300 mg per dose and a minimum interval of one and a half hours. The dosages of Demerol in the two cases cited in some detail ranged up to 3,180 mg a day in one case, and up to 2,850 mg in the other.

SUMMARY

A case is reported wherein a patient took 1250 mg of Demerol with suicidal intent. This resulted in mild toxic symptoms, and there was prompt recovery without sequela.

Demerol is a drug frequently used by allergists. Animal experimentation has shown the relative low toxicity of Demerol, but this is the first case in so far as could be found in the literature in which a human being took such a large quantity in a single dose. The relative mildness of the toxic symptoms sustained suggests that Demerol, in addition to being an efficient drug, is likewise a relatively safe drug to use in patients in whom suicidal tendencies may be suspected.

Suit 517 Brown Bldg.

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PRACTICES AVAILABLE

Any allergist of the College interested in locating in California please contact the Secretary's office, 423 La Salle Medical Building, Minneapolis 2, Minnesota, for particulars.

* * *

Any young man who would be interested in going to Texas and locating in a good-sized city to take over the practice of a Fellow of the College specializing in allergy, will please contact the Secretary of the College at once for details.

This Fellow is a member of the reserves and is expecting to be called back to regular duty in the near future. To insure that his patients receive the proper treatment in his absence, he would be willing to make an agreement to turn over his practice for a small percentage based upon the volume that it is now. Any allergist who is able to maintain it at a larger level than it is now would keep the extra revenue. He would also be willing to sell the entire practice to another allergist with the understanding that when the emergency is over, he would not locate in the same city.

SKIN REACTIONS OF SURFACE ANTIGENS AND BACTERIAL RESIDUES

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THE following study is part of a general investigation of skin reactivity among tuberculous patients. Previous reports[1-5] have given details of tuberculin reactivity under various conditions in tuberculosis, and pollen hypersensitivity in those patients who had both pollinosis and tuberculosis. The availability of new, and possibly improved, bacterial antigens led to this study.

METHODS AND MATERIALS

The bacterial antigens used* were surface antigens and bacterial residues of common organisms. The surface antigens (immunogens) are obtained by rapid extraction and sedimentation of living bacteria grown on solid media. They are presumed to contain the soluble specific substance (haptinic carbohydrate) and theoretically should be an improvement over the usual bacterial filtrates. The remaining bacterial bodies, after removal of the surface antigens, are suspended to make the bacterial residues. The two fractions mentioned were obtained from each of the following bacteria: (1) N. catarrhalis; (2) E. coli; (3) Friedlanders' bacilli; (4) H. influenzae; (5) pneumococci 1, 2, and 3; (6) Staphylococcus albus and aureus; (7) Streptococcus hemolyticus and non-hemolyticus. Controls from the culture media (two varieties) were available.

All patients tested had active pulmonary tuberculosis. Most of them were afebrile and ambulatory, a few (twelve) toxic and febrile (101° to 103° F.). Eleven children, ages three to eleven years, were tested with N. catarrhalis and E. coli.

The injections were intracutaneous, with a routine volume of 0.05 cc as delivered by a 1 cc tuberculin syringe with 26-gauge needle.

RESULTS

Tests of the seven immunogens and seven bacterial residue suspensions were done first on twenty ambulatory tuberculous patients. In fifteen minutes all tests showed a slight increase in the size of the wheal, with variable erythema and no pseudopods. The residues gave slightly larger reactions than the immunogens, but in each group all reactions were closely alike in size.

At eighteen to twenty-four hours the tests revealed an area of edema and induration varying in size from 0.1 cm to 2 cm in diameter, with variable surrounding erythema and no pseudopods. The staphylococcus

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*These materials were generously furnished by Parke, Davis & Co.

and E. coli materials gave the largest reactions, the pneumococcus the smallest.

The average diameter of the induration for each of the antigens at eighteen hours follows:

	Immunogen	*Suspension*
N. Catarrhalis	0.5 cm	0.5 cm
E. coli	0.9 cm	1.1 cm
Friedlanders'	0.6 cm	0.8 cm
Staphylococcus	1.0 cm	0.8 cm
Streptococcus	0.3 cm	0.3 cm
H. influenzae	0.6 cm	0.8 cm
Pneumococci	0.1 cm	0.3 cm

At forty-eight hours the tests had faded, leaving a small area of induration from 0.1 cm to 0.4 cm in diameter, with almost no erythema. At sixty hours practically no reaction remained.

Following the above tests with both immunogens and residues, twenty-nine other ambulatory tuberculous patients were tested with three to six of the immunogens. The reactions were closely similar to those described above. Twelve patients with fever from 101° to 103° F. were tested with immunogens. Most of these gave reactions identical with the first group; a few who were moribund gave very small reactions.

Tests on eleven tuberculous children (age three to eleven years) with N. catarrhalis and E. coli immunogens gave reactions quantitatively and qualitatively the same as the adults.

Tests with extracts of media were uniformly negative.

DISCUSSIONS

Surface Antigens.—It was to be expected that the immunogens, if they contained specific carbohydrates in sufficient concentration, might give immediate reactions in suitable patients. No such reactions could be noted. Whether this was due to insufficient antigen or lack of proper reactivity in the patients cannot be stated. The presence of definite delayed reactions with the immunogens indicates clearly that they contain considerable quantities of antigens similar to those in the bacterial residues.

Bacterial Residues.—The reactions with these materials appears similar to those obtained with vaccines. The parallelism, both quantitatively and qualitatively, between the immunogen and bacterial residue reactions seems to indicate that both contain the same antigen or antigens.

Are the reactions specific? The fact that patients react to all of these bacterial products with but slight quantitative differences, raises the question of specificity. Are these reactions the result of earlier infections

(Continued on Page 577)

Editorial

The opinions expressed by the writers of editorials in the ANNALS *do not necessarily represent the group opinion of the Board or of the College.*

ALLERGIC ASPECTS OF RHEUMATISM AND ARTHRITIS

The newly established Rheumatism and Arthritis Committee of the American College of Allergists is functioning under the able leadership of Dr. George E. Rockwell. Although multiple factors enter into the production of the rheumatic and arthritic diseases, the Committee believes that the allergic aspects are sufficiently important clinically to deserve special attention. Recognizing this, the Board of Regents of the American College of Allergists has decided that the subject for next year's panel discussion will be "The Allergic Aspects of Rheumatism and Arthritis."

While there is much evidence in the literature that allergic mechanisms are responsible for many of the clinical manifestations of the rheumatic and arthritic diseases,[8,11,14,16] leading rheumatologists have been reluctant to accept this concept, particularly as it applies to the arthritic diseases. But many rheumatologists as recently as 1948 insisted that endocrine dysfunction was in no way involved in the production of clinical manifestations of rheumatism and arthritis.[6] Today they are on the endocrine band-wagon. Perhaps in time these rheumatologists will become more interested in allergic aspects of these diseases, since there is increasing evidence that allergic reactions are modified in a favorable direction through the use of endocrine agents, ACTH and cortisone.[1-7,9,10,12,13]

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LETTER TO THE EDITOR

To the Editor:

The closed-minded approach to the flat contradictions in the analysis of observed food-allergic reactions is instructively illustrated in the symposium on cottonseed sensitivity printed in the January-February, 1950, issue of the ANNALS OF ALLERGY.

The symposium includes reports of the two kinds of familial sensitivity to the cottonseed allergen—the reaginic and the nonreaginic.

The reaginically sensitive cases were exclusively reported by McGrath, Loveless and Mitchell, all of whom *selected* their cases on the basis of positive *cutaneous* reactions.

The nonreaginically sensitive cases were exclusively reported by Randolph and Sisk, and indirectly by Rowe, all of whom selected their cases on the basis of *ingestion tests,* the skin-tests being *subsequently* found negative.

The former group, after proving without question, by means of the skin tests, the specific sensitivity of all their selected patients to the cottonseed allergen, could produce no clinical symptoms in any of them through ingestion of cottonseed oil.

The latter group, after proving without question, by means of the simple ingestion test, the specific sensitivity of their selected patients to cottonseed oil, could elicit no specific cutaneous reaction with the cottonseed allergen.

Each group ignores the findings of the other and Loveless urges the adoption of "the more scientific study" which she has outlined in her "general principles"—presumably referring to her quantitative estimation of the reaginic sensitivity and concentration (dosage) of the cottonseed allergen.

However, one may observe that in "scientific" study the matter of

quality is sometimes a much more important consideration than that of quantity. All the quantitative studies of precipitin in serum sickness had failed to solve the immunologic etiology of that condition until Karelitz, using a *different technique* (Voss), proved the antibody of serum sickness to be *qualitatively* different from precipitin and so upheld the original judgment of Von Pirquet and Schick. The partizans of precipitin will not be permitted in the future literature of serum-sickness to ignore the reports of Karelitz; and I venture to predict that future students of food-allergy will give due attention to the convincing results of the ingestion-tests reported by Rowe and by Randolph and Sisk with cottonseed oil.

In fact, Loveless and her group have been misled in their study by their very disregard of the great body of evidence which has been obtained with the ingestion procedure and reported by such competent clinicians as Vaughan, Peshkin, Rinkel, Davison, Milo Meyer and others.

They all overlooked the fact that nonreaginic sensitivity to cottonseed is so uncommon that they were likely to find no such case among their reaginically sensitive patients. Not recognizing the distinction between atopic and nonreaginic allergic symptoms, Loveless failed to notice that the symptoms which she observed in her atopic patients following ingestion of cottonseed protein were *atopic* (sneezing, conjunctival itch, itching of mouth and throat, slight nausea). The relatively large doses that were required suggest that the effect was comparable to the familiar constitutional effect of overdosage of pollen extracts, plus direct contact with the alimentary mucous membrane.

Nonreaginic constitutional symptoms, on the contrary, sometimes follow the ingestion of extraordinarily minute quantities of food protein. I have reported[1] a well controlled instance of sensitivity to sugar-cane protein in whom the characteristic symptom, vertigo, was elicited by daily ingestion of 0.00000003 mg of that protein.

Hence, one need not be surprised when the presumably small quantity of protein in cottonseed oil causes *nonreaginic* allergic symptoms, as it unquestionably does.

Part of the onus of the contradictory nature of the reports must be borne by those who have observed the nonreaginic allergic reactions without realizing and pointing out their separate etiology with reference to the atopic category. These men have faithfully recorded the absence of skin-sensitizing reagins in obviously (even violently) allergic persons, yet apparently without being able to digest the logical conclusion forced by that observation.

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Progress in Allergy

ANTIHISTAMINIC AGENTS

A Review

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PYRIBENZAMINE

Of the many papers concerned with the effect of Pyribenzamine on anaphylactic shock in guinea pigs and on various manifestations of histamine toxicity, only those of particular interest have been chosen for review. Koepf et al[293] showed that 100 mg. doses given to dogs orally, daily, for one year, and two given 50 mg. daily for the same period, had no effect on general behavior. There was no evidence of kidney or liver dysfunction or granulocytopenia. Three human volunteers given 150 mg. daily for eighty days showed no changes in well-being, body weight, kidney or liver function, blood pressure, or blood picture. Five allergic individuals given 100 to 400 mg. daily for six to 275 days showed no significant changes in blood pressure. Gross and Meier[294] showed that the severity of chemosis produced, in rabbit's eye, by the application of a 10 per cent mustard oil solution was reduced by the prophylactic administration of 1 to 10 mg./kg. intravenously or subcutaneously. Antistine (5 to 10 mg.) and Phenergan (20 mg.) had similar, but lesser, effects. The vascular reactions produced by mustard oil were not alleviated while the prophylactic administration of rutin (50 to 100 mg.) inhibited the vascular reactions but was without effect upon the chemosis. In the experimental work so far reported, histamine was either injected or aerosolized. A new approach by Kellner et al,[295] consisted of the use of subcutaneous pellets containing Pyribenzamine (500 mg.), beeswax (2.0 gm.), which protected thirteen of fourteen guinea pigs against lethal doses of histamine for seven to sixteen days.

In dogs, the administration of Witte's peptone (1 gm./kg.) is almost invariably fatal. According to Davis and Haterius,[296] the prophylactic administration fifteen minutes previously of 2 mg./kg. atropine, or of Pyribenzamine, 10 mg./kg., protected, in the first place, five of six, and in the second, all of six dogs so treated. The blood pressure which initially fell rapidly in all animals rose faster and higher in those given Pyribenzamine than those given atropine alone, or the combination of both drugs. From this, the authors conclude that histamine apparently, and to a lesser extent, acetylcholine, are both involved in peptone shock.

The use of antihistaminic agents in the treatment of allergic conditions in animals should, perhaps, be mentioned at this point. Rawson[297] reported that the oral administration of Pyribenzamine (25 mg.), one to four times daily to 160 dogs with eczema, urticaria, asthma, edema, conjunctivitis, resulted in excellent effects in 43 per cent, improvement in 29 per cent, and negative effects in 27 per cent. Intravenous injection of 300 to 800 mg. in sterile saline solution into eleven horses with laminitis and nine with azoturia, food allergy, bee stings and pulmonary edema, gave excellent results in fourteen, improvement in four, and no results in two. Seventeen of eighteen cows suffering from mastitis, diarrhea; ketosis, retained placenta, septic metritis, and food allergy, showed good results. In dogs, the side reactions were vom-

iting and nervousness and in large animals, nervousness and muscular tremor, salivation and other side effects lasting ten to thirty minutes.

Because of the histamine effects of severe burns seen in humans, Gunnar and Weeks[298] induced burn shock in twelve of fourteen rabbits, seven receiving large doses of isotonic sodium chloride solution every two hours in quantities sufficient to give the animals 1 mg./kg. of Pyribenzamine. There was no decrease in severity, the delay of onset nor inhibition or progression of the shock noted in the treated series. On the other hand, areas of erythema on the skin of rabbits produced by burns, histamine phosphate, skin wheals and intradermal injections of turpentine were, according to Weeks and Gunnar,[299] reduced in an average of 43, 29 and 47 per cent, respectively, by prior administration of 4 mg./kg. Pyribenzamine, which caused irritability, restlessness and, if given rapidly, convulsions in the test animals.

Pyribenzamine is so widely used that its toxic reactions must be given special emphasis. According to Brown,[300] who gave 50 mg. tablets to a group of 100 patients at four-hour intervals and ten 50 mg. tablets within twenty-four hours to another group of fifty-six patients, while the third group of 102 patients received five placebos in twenty-four hours, the occurrence of side reactions in the three groups were as follows: drowsiness, respectively, 37, 48 and 30 per cent; headache, 26, 36, and 42 per cent; nausea, 17, 23, and 8 per cent; dizziness, 24, 41 and 15 per cent; nervousness, 13, 21, and 15 per cent; oral dryness, 29, 45, and 30 per cent, and insomnia, 12, 23 and 6 per cent. It will be noted that however much the patients taking the placebo medication complained of side reactions, there is no doubt whatsoever that the patients given ten 50 mg. tablets in twenty-four hours reported the largest amount of untoward effects.

According to Wolfson,[301] a fifty-year-old male, taking Pyribenzamine for a mild dermatitis suddenly developed a painless urinary obstruction. With the discontinuation of the drug the obstruction promptly abated, a subsequent trial reproducing the symptoms. Investigations carried out on dog intestines and uterus demonstrated the presence of a spasmogenic property in Pyribenzamine and other antihistamines. It was concluded that the urinary symptoms were the result of such a property in the drug given. Dermatitis may follow Pyribenzamine ingestion. Epstein[302] reported on two patients; the first, responding with a pityriasis-rosacea-like reaction; and the second, with an erythematopapular dermatitis following the ingestion of Pyribenzamine, reappearing after subsequent administration of the drug. Pipes[303] reported on a ten-year-old boy, who on two occasions following Pyribenzamine in the 25 mg. dose twice daily presented a coarse, scattered papular, very pruritic dermatitis within twenty-four hours, the condition progressing to vesiculation and pustulation. According to a well-controlled study by London and Moody,[304] Pyribenzamine may cause acute urticaria. Blanton and Owens[305] reported granulocytopenia as probably due to Pyribenzamine following eight weeks of treatment. Provocative doses were not given. A patient reported upon by Cahan et al[306] developed a sore tongue, sore mouth and agranulocytosis following Pyribenzamine ingestion. She had, however, until one week previously received ergotrate and empirin compound. Lott, Krug and Glenn[307] feel certain that the administration of 100 mg. of Pyribenzamine every four hours to a twenty-year-old girl resulted in acute delirium resembling a psychosis, appearing forty-eight hours after the initiation of Pyribenzamine treatment, and improving within four hours after its discontinuation, to disappear completely in twelve hours. The patient was completely normal in three days, there being no other explanation for her reaction. Ross[308] reported on four patients, who on 75 to 100 mg. daily Pyribenzamine for two to thirty-one days complained of blurred vision, with depressed accommodation in two, refractive changes in two, and diffuse corneal edema with an increase in corneal relucency in one. The pupillary reflexes were normal. The changes all disappeared within two weeks after the drug was discontinued. According to the Council on Pharmacy and Chemistry,[309] Pyribenzamine

is described as useful in preventing histamine-induced smooth muscle spasm and in treating patients with urticaria or seasonal allergic rhinitis with or without bronchial asthma. It is stated that side reactions of varying severity occur in 30 per cent of the patients treated and that therefore the smallest dose of the drug which will control symptoms should be given. In the absence of side effects, 100 to 150 mg. four times daily should be given.

Pyribenzamine has been used in the treatment of a number of skin conditions, both orally and by application. When patients were given 25 to 50 mg. three to eight times daily, Baer et al[310] found that satisfactory anti-pruritic effects occurred in 10 per cent of 124 patients with itching dermatoses other than urticaria. In this latter condition, the pruritus was controlled and the clinical lesions reduced in approximately 75 per cent of the patients. In twenty-five patients, the side effects noted included drowsiness, dizziness, excitement, sweating, headache, polyuria, pyrosis, diplopia, a feeling of cold, reduction of potency, and difficulty in urination. In some cases when the dose was reduced to 12.5 to 25 mg. three times daily and gradually increased, tolerance occasionally resulted. In a second report, Sulzberger et al[311] used Pyribenzamine locally in ninety patients with a variety of dermatoses, the two to five per cent cream being applied three to four times daily. Twenty-five of forty patients with atopic dermatitis were improved, with two showing a transient lessening of pruritus, while thirteen were made worse. Of eighteen patients with dermatitis eczematosa, fourteen remained the same or became worse, with two improving in the pruritus and clinically, and two showing transient improvement. Only eight of sixteen with circumscribed neurodermatitis improved, with four showing no change or deterioration, and four more being held temporarily. Three of five patients with pruritus ani or vulvae showed no improvement, two being helped for a short period of time. Nine of eleven patients with psoriasis, seborrheic dermatitis, dermatitis herpetiformis, circumscribed scleroderma, lupus erythematosus, nummular eczema or erythema multiforme, were unchanged or made worse, with two showing temporary improvement. Of the five patients in whom the untoward reactions necessitated discontinuation of the drug, two presented an allergic eczematous contact-type sensitization, while three gave evidence of systemic side effects, probably due to absorption. Frankfeldt[312] used both oral and topical Pyribenzamine in ninety patients with pruritus ani. Of these, four patients were forced to continue the drug, one because of profound drowsiness, one because of cardiac palpitation, and two because of a generalized maculo-papular eruption. Of the others, fifty-seven experienced relief of itching, thirty-three experiencing no relief and sixteen not returning after the first visit. Eleven of those who reacted favorably to the oral medication were able to control the pruritus by local application of the 2 per cent ointment. Aaron, Peck, and Abramson[313] treated twenty patients with various pruritic dermatoses, with the 5 to 10 per cent solution of Pyribenzamine administered iontophoresis. The patients usually received six to ten daily treatments, each lasting five minutes, using a 4 to 10 ma. current. Ten patients obtained complete remission, and all the others experienced either relief during the course of treatment or clinical improvment. The first few treatments caused a burning sensation and increased erythema which decreased as the treatment continued. Experimentally, two hours after a 10 per cent solution of Pyribenzamine had been introduced into the skin by iontophoresis, patch tests with concentrations of turpentine, which caused vesicles, were without effect. Rogers[314] also used the 2 per cent Pyribenzamine ointment as well as the oral medication in twenty-seven patients with skin disorders. There was little or no improvement in patients with atopic dermatitis or pustular psoriasis. Either relief of itching, improvement or complete recovery was produced in patients suffering from seborrheic dermatitis, exudative discoid lichenoid chronic dermatitis, pruritus ani, nummular eczema and contact dermatitis. In several patients, flare-ups were produced

immediately, and in one patient, an irritating effect after the drug had been used for several weeks.

Pyribenzamine has been considered in the prophylaxis and treatment of poison ivy dermatitis. Twedall and O'Connor[315] discovered that the administration of 100 mg. one hour prior to the application of an acetone extract of Rhus toxicodendron to the forearm, and 50 mg. at the time of application and every four hours thereafter for twenty-four hours did not affect the vesiculation of any of five allergic subjects. One patient reported some relief of the burning and itching. In a series of 280 patients with allergic and other pruritic dermatoses, Kesten[316] observed that oral doses of 50 to 100 mg. four times daily achieved complete relief of symptoms in only forty-seven. These included six of eight patients with serum sickness, one of four with dermographism, and thirty of 166 with urticaria. In 144 patients there was a suppressive action, but in thirty-six patients, untoward reactions, including nervousness, gastrointestinal disturbances, palpitations and drowsiness, necessitated the discontinuation of the drug.

According to Silverman,[317] Pyribenzamine completely relieved the pruritus due to chickenpox and measles. The author reports that after two to three doses there is complete relief and the number of secondary skin infections is materially, if not entirely, reduced as a result of Pyribenzamine therapy.

In a carefully controlled study, Zondek and Bromberg[318] used Pyribenzamine inunctions for pruritus vulvae. The patients, sensitive to endogenous hormones, had not responded to oral administration of the drug. In order that the interpretation of the effects of Pyribenzamine might be accurate, each of fourteen patients was first treated by local application of full strength glycerine for one week and by inunctions of 3 per cent procaine in glycerine for a second week. During the third week, the solution of Pyribenzamine and glycerine was applied at eight-hour intervals. In three of the patients, allergy to endogenous hormones was the etiological factor; in four there was a vitamin deficiency, in two diabetes mellitus, trichomonas vaginitis in two, vulval vaginal mycosis in one, psychoneurosis in one, and menopausal changes in one. Of these, temporary relief of itching was obtained in seven patients, with six improving and one not responding. The relief and improvement were observed in the cases due to allergy, diabetes, vitamin deficiency and vulval-vaginal infections. The effect did not persist beyond the cessation of treatment.

The use of Pyribenzamine interchangeably with Benadryl in the treatment of penicillin sensitivity has been described in the section dealing with the latter drug. In the treatment of sensitivity to streptomycin, Cohen and Glinsky[319] gave Pyribenzamine to six of fifty-five patients with a generalized maculopapular erythematous rash with mild or extreme pruritus and eosinophilia due to streptomycin (2 gm. daily). The pruritus responded promptly, the rash being self-limiting and lasting five to ten days. Urticaria, when present, did not disappear until six weeks after the streptomycin therapy had been stopped. An exfoliative dermatitis which developed on the fortieth day of treatment in one patient following a rash which appeared on the twenty-sixth day was not relieved by antihistaminic agents and necessitated streptomycin withdrawal. The rash appeared in one of sixteen patients treated with 1 gr. of streptomycin daily and in two of sixty-six treated with 0.5 gm. daily.

In chloromycetin sensitivity as manifested by a giant urticaria, Pyribenzamine may lessen the symptoms and decrease the itching in about two hours, as demonstrated by a patient described by Sachs.[320] There was no history of allergic reactions to drugs or any other type of allergy. Chloromycetin was administered in a total dose of 9 gm. over a five-day period for symptoms of typhoid fever. Seventeen days later, a second course of chloromycetin therapy was instituted, and in eight days the patient responded with erythematous patches, which in twenty-four hours were accompanied by swelling of the eyelids, hands and ankles, as well as a sore throat, extending over the trunks, arms, buttocks, thighs and hands.

The use of Pyribenzamine in intravenous pyelography was described by Getzoff,[321] who administered the drug before injecting Diodrast. No reactions occurred in fifty non-allergic patients so treated, with general sensitivity reactions occurring in only four of twenty-two allergic individuals. The patients received Pyribenzamine (100 mg.) and also epinephrine.

In the treatment of allergic conditions, the following results were reported by Friedlaender and Friedlaender.[322] In 200 patients treated with 50 to 200 mg. orally, children taking 25 to 50 mg., both four times daily, the drug caused partial or complete relief of symptoms in approximately two-thirds of the patients in each of the following categories: vasomotor rhinitis, 108; grass and ragweed hay fever, ninety-eight; urticaria and angioneurotic edema, twenty-four; atopic and contact dermatitis, twelve. It relieved the pruritus associated with other conditions and controlled the symptoms arising out of skin tests and desensitization treatment. It gave no relief in 30 patients with non-seasonal bronchial asthma. In 27 per cent of the cases there were side effects, the most common being gastrointestinal symptoms, drowsiness and vertigo. In the group studied by Arbesman et al[323] there were 495 patients suffering from 565 allergic conditions, in whom relief was obtained in 411. The dose for children was 50 to 200 mg. and that for adults, 100 to 400 mg. daily. Relief was apparent in fifteen to twenty minutes and lasted for one to twelve hours. No relief was seen in patients with migraine, histamine cephalalgia, Ménière's syndrome, dermatitis venenata, psoriasis or acne rosacea. The report differs from that previously given in that six of twelve patients with bronchial asthma due to grasses were reported as relieved and twenty-eight of sixty-two patients with extrinsic bronchial asthma. Very definite relief of symptoms was seen in thirty of thirty-four patients with allergic rhinitis, in eighty-nine of 106 patients with ragweed pollen hay fever, and 100 of 138 patients with extrinsic non-seasonal allergic rhinitis; in seventeen of thirty-five patients with intrinsic allergic rhinitis and in forty-four of forty-seven patients with acute urticaria. All of twenty-three patients with chronic urticaria following penicillin therapy were relieved in three days to three weeks. The conjoint studies of the Committee on Pharmaceuticals and Medicaments (American Academy of Allergy)[324] listed the drug as improving 59 per cent of 277 patients with non-seasonal vasomotor rhinitis, 54 per cent of 104 patients with seasonal vasomotor rhinitis, 31 per cent of 211 patients with non-seasonal asthma, and 45 per cent of eleven patients with seasonal asthma. It also improved 77 per cent of 121 patients with acute urticaria, 78 per cent of ninety-seven patients with chronic urticaria, 78 per cent of twenty-three patients with dermographism, 57 per cent of twenty-one patients with pruritus, twenty-two per cent of nine patients with eczema, 60 per cent of five patients with histamine headache, 51 per cent of fifty-nine patients with atopic dermatitis, 20 per cent of five patients with eczematous dermatitis, 57 per cent of seven patients with unclassified dermatitis, 20 per cent of five patients with Meniérè's syndrome, 28 per cent of seven patients with gastrointestinal allergy; 40 per cent of five patients with cold allergy; 1 per cent of three patients with visual disturbances; and 17 per cent of six patients with migraine. Of 978 patients treated, side effects were noted in 168, with sleepiness affecting sixty-one; nausea, twenty-six; dizziness, seventeen; headache, twelve. Other side effects listed included flushing of the skin, tachycardia, wakeful excitement, difficulty in accommodation, weakness, chills, urticaria, vomiting, early menses, dry mouth, nervousness, paresthesias, gastrointestinal cramps, and symptoms of diabetes insipidus.

Feinberg and Friedlaender[325] studied 503 patients whose conditions included chronic and acute urticaria, atopic dermatitis, dermographism, penicillin and sulfonamide reactions, pruritus vulvae, chronic allergic rhinitis, seasonal hay fever, asthma, chronic headache, and gastrointestinal allergy. Twenty-one of twenty-seven with chronic urticaria and angioneurotic edema experienced relief from itching and reduc-

tion in lesions as long as the drug was continued. In other conditions, 50 mg. doses led to symptomatic relief for several hours in most cases. The symptoms of the usual course and duration returned when the drug was discontinued. The side reactions listed above were noted.

In a comparative study, Arbesman et al[326] found that 72 per cent of fifty-four hay fever patients, and 41 per cent of twenty-six asthmatic patients were relieved of their ragweed pollen sensitivities when Pyribenzamine was given alone. Another group of twenty-seven patients with hay fever and eighteen with asthma received pre-seasonal desensitization, reporting 67 per cent relief for the first group; and 56 per cent for the second. A third group of 242 patients were desensitized and given Pyribenzamine, those with hay fever reporting 95 per cent relief. The patients took 400 mg. daily and the maximum doses of pollen extract which could be tolerated. On the other hand, Fuchs et al[327] reported that there was no statistically significant difference in the relief afforded hay fever patients by treatment with ragweed pollen extracts, Pyribenzamine, or the combination. The drug was given alone to a group of forty patients, 50 mg. doses three times daily, and to thirty-two patients, twenty minutes before each pollen injection. In a forty-two-day period, 65 per cent of those receiving Pyribenzamine alone, 75 per cent of those receiving pollen extract alone, and 72.6 per cent of those given both showed no or slight signs of symptoms. In 17 per cent there were complaints of the usual side reactions. Gorin[328] reported on Pyribenzamine alone in the treatment of hay fever of at least three years' duration in thirty-eight children, given 25 mg. three times daily. Six were definitely improved, seven moderately improved and twelve obtained no relief. Discontinuation of the medication in the group improved caused the prompt return of symptoms. In one child, extreme drowsiness was noted. According to Henderson and Rose,[329] the most favorable results following the use of Pyribenzamine occurred in the cases of hay fever, forty-one of sixty-one treated cases being kept free of symptoms. In twenty-two cases, patients with hay fever and bronchial asthma, the drug controlled the hay fever in fourteen and was not effective in the remaining eight. Ten patients were relieved of their asthma, while twelve more were not affected. In fifteen cases of chronic bronchial asthma associated with infection, relief occurred in three, but actual exacerbation of symptoms occurred in three others. In two patients, mild attacks of asthma could be aborted but severe attacks were unaffected. There was some relief in thirteen of twenty-one patients with non-seasonal allergic rhinitis. Patients who were also given a choice of Antistine and Benadryl preferred Pyribenzamine by a large majority.

The use of Pyribenzamine (0.5 per cent solution) applied topically was studied by Schwartz and Leibowitz[330] in fifty-nine seasonal and thirty-five non-seasonal hay fever patients, with partial or complete relief occurring in 83 per cent of the seasonal and 75 per cent of the non-seasonal patients. Of these, 45 per cent complained of slight, and two of extreme burning sensations, 20 per cent obtaining no relief whatsoever. According to Brem and Zonis,[331] however, the application of 0.5 per cent solution locally brought about successful antihistaminic and local anesthetic effects in seventy-six of eighty-one patients with allergic rhinitis, with only two patients being unable to tolerate the drug because of aggravation of allergic symptoms and head pain. In some patients there was transitory stinging of the otopharynx, and mild paroxysms of sneezing. Relief usually lasted one to twenty-four hours.

The application of an aerosol of Pyribenzamine (2 per cent) solution was studied by Feinberg and Bernstein,[332] who reported that its use every two to three hours benefited twenty-seven of thirty-four patients with nasal congestion due to seasonal hay fever or allergic rhinitis. Only those patients who could not obtain results or tolerate oral antihistaminic therapy were treated. The effects of the solution were

frequently not observed until after several applications. Of fifty-seven patients with moderate bronchial asthma, most of whom had not responded to oral medication, ten were effectively relieved and seventeen slightly so. Patients with spasmodic cough without dyspnea responded better than those with true asthma. Twelve of sixteen patients reacted favorably. Although less effective than aerosol epinephrine solutions, the Pyribenzamine solution was considered to be less toxic.

Nasal iontophoresis with Pyribenzamine has been reported upon by Fenton and Huffman,[333] who treated patients with seasonal and perennial hay fever twice weekly (3 to 7 ma. for 8 minutes each nostril) using nasal packs saturated with 2 to 5 per cent solutions. Temporary dizziness occurred in 50 per cent and drowsiness in 20 per cent of twenty patients, in whom immediate improvement was obtained in eight, with two being relieved for eight hours and six for forty-eight hours. In addition, eleven patients with perennial hay fever were treated for four to eight weeks and showed 75 per cent benefit in five subjects, in two of whom relapses occurred in two weeks. Moderate improvement of fairly long duration occurred in the other six. In five patients, in whom other forms of antihistaminic therapy had failed, Aaron[334] found that iontophoresis with Pyribenzamine (5 per cent aqueous) brought relief for one to four days. Of two additional patients with allergic perennial rhinitis, one obtained relief for more than seven days, but another for only three hours. One with vasomotor coryza complained of dryness of the nose lasting one day.

As with the other drugs reviewed, Pyribenzamine has been used for a number of miscellaneous conditions. Since these may in time prove to be more important than perhaps those due to allergy, they are, as a matter of record, listed. According to Foster and Hanrahan,[335] a homograft in a Negro female with a full thickness skin defect appeared to be viable at the end of sixty and ninety days. Although it was not possible to ascertain whether the original graft had been replaced by tissues from the host, nevertheless, it is felt that the administration of Pyribenzamine for homografting may have been responsible for the successful result.

Moseley[336] used Pyribenzamine (1 per cent aqueous) in two portions of 10 c.c. each as a mouth wash and gargle to provide oral and pharyngeal anesthesia for thirty patients prior to gastroscopy. The drug diminished the gag reflex and permitted the passage of a gastric tube in five patients. A 1 per cent solution was used fifteen minutes before eating to relieve discomfort in food ingestion in two patients with aphthous stomatitis. In two patients with severe sore throats and one with painful caries, topical application relieved the condition, as it did in two patients with painful hemorrhoids, who used the drug in an ointment base. The anesthetic effect lasted one to two hours. In normal individuals, it was discovered that a mouth rinse (0.5 to 1 per cent) used for one to two minutes caused a bitter taste, which disappeared in thirty to forty-five minutes with anesthesia coming on in four and lasting up to thirty minutes.

Although the use of antihistaminics in tuberculosis has already been noted as favorable, Guy[337] found no significant alteration in the skin reaction of five individuals to whom 150 mg. of Pyribenzamine was given orally one hour before, and 650 mg. in divided doses forty-eight hours after, the intracutaneous injection of 1:1000-1:100,000 old tuberculin skin tests. There was no significant alteration in the reaction. Friedman and Silverman[338] found that oral doses (60 mg. daily) for four days had no inhibiting effect on the tuberculin reaction of forty-three children. In doses of 240 mg. daily for four days there was no effect on tuberculin reaction in ten children. They suggest that in the routine tuberculin testing, one need not be concerned as to whether the patient is receiving coincident Pyribenzamine or any other antihistaminic medication.

Green and Kline[339] found Pyribenzamine useful in the treatment of varicose ulcer.

In eight patients in whom the ulcers varied from 2.5 by 2.5 cm. to 10 by 10 cm., healing occurred in four to ten weeks, with pain and edema subsiding rapidly in eight patients given 200 to 500 mg. daily. The patients discontinued all local treatment, excepting a small amount of ointment and zinc stearate dusting powder. All of the patients had previously been treated unsuccessfully with ointments and two had had injection therapy. Observation of five patients four to thirteen months later showed no evidence of recurrence.

McEachern[340] administered doses of Pyribenzamine (50 mg. three times daily) to a patient with angina pectoris who had developed contact dermatitis. The irritation was relieved in four to six hours and the drowsiness seen initially disappeared in four to five days. The patient reported that his anginal pain was alleviated and the drug was therefore continued twice daily and reduced in dosage. In seven of eight additional patients treated with Pyribenzamine, the angina pectoris was definitely reported as improved, as measured by exercise tolerance.

Hoffman[341] gave 50 mg. three times daily to forty pregnant patients, who suffered from albuminuria and/or hypertension and in all of whom salt and fluid intake had been restricted. Of these, eleven of thirteen with albuminuria became albumin-free and two were not benefited. Ten of twelve with hypertension showed a significant drop in blood pressure, with two remaining unchanged, and twelve of fifteen suffering from both conditions showed a definite drop in blood pressure, while eleven became albumin-free. In three patients neither condition was improved, one of these presenting a still-born and another, a premature infant.

According to Perry and Horton,[342] in twelve of fourteen patients in whom histamine caused increase in gastric acidity, Pyribenzamine given in doses of 100 to 400 mg. daily for one to eight days proved to be without effect. In histamine-induced headaches, it was considered that Pyribenzamine lessened the intensity and duration of the pain in three of four patients, in the fourth of whom no headache developed. In three patients hypersensitive to cold, both Pyribenzamine and Benadryl gave symptomatic relief, reducing the severity and duration of the urticaria and edema. Rubin and his co-workers[343] noted that two patients under treatment for solar urticaria found that the areas of skin normally exposed were more tolerant to sunshine than those usually covered. The observation was confirmed by exposing the whole body surface of one patient to the irradiation of an artificial sunlamp daily with Pyribenzamine given in 50 mg. doses three times daily. Fourteen days after the drug had been discontinued, sensitivity tests to light on both exposed and unexposed parts of the abdomen showed that the irradiated portions had acquired a tolerance more than 200 times greater than the covered parts. This effect was due pigmentation and/or thickening of the horny layer. The authors feel that no immunological desensitization mechanism was involved.

Morrow[344] preferred Pyribenzamine to Benadryl in the treatment of 241 patients with various dermatoses. Only ten to 12 per cent of those on Pyribenzamine suffered side effects, namely drowsiness, which occurred, however, in 50 to 60 per cent of the patients treated with Benadryl. Headache, nausea and diuresis occurred to an equal extent in both groups. Patients suffering from penicillin reactions, pruritus ani and vulvae, acute and chronic urticaria, erythema multiforme, poison oak dermatitis, dermatitis venenata, and dermatitis medicamentosa, as well as patients with seborrhea showed some improvement. Treatment was less successful in patients with atopic dermatitis, nummular eczema, post-scabetic pruritus, stasis dermatitis. Dermatitis herpetiformis, jaundice pruritus, neurotic excoriations and actinic dermatitis were not improved. In several patients, both drugs were given simultaneously with no added benefit.

Kells[345] recommends Pyribenzamine as an adjunct in the control of morphine withdrawal symptoms, administering the daily requirements of morphine in six

doses every four hours, reducing by 10 per cent each day and starting Pyribenzamine (50 mg. four times daily) when the morphine dosage is one grain. The Pyribenzamine is continued for three days after the last dose and is needed thereafter to relieve withdrawal symptoms. No sedation is used excepting sodium bromide. The author believes that Pyribenzamine neutralizes an anti-morphine substance responsible for abstinence symptoms.

Sherry[346] found that in five patients with Hodgkin's disease, who responded to nitrogen mustard, the fever and glandular enlargement responded excellently to six daily doses of Pyribenzamine. A patient who suffered a second flare-up after a course in nitrogen mustard responded similarly to the drug.

According to Murray,[347] Pyribenzamine will cure the common cold as observed in 397 and 494 patients treated with Pyribenzamine every four hours. Some patients reported relief after the first two tablets, and 110 had no symptoms after forty-eight hours. In 204 patients, the cold disappeared in one to three days, and in eighty-three, the cold was greatly improved. Only seven patients lost time from work because of colds; twenty-two of ninety-seven patients who did not respond to treatment suffered untoward reactions, dizziness affecting seven, drowsiness, six, severe headache, five, and digestive disturbances, four.

Schiller and Lowell[348] discovered that Pyribenzamine did not influence the pulmonary response to inhaled extract of pollens, as measured by change in vital capacity in three asthmatic individuals. They feel, therefore, that neither acetycholine nor histamine are determining factors in the production of pollen-induced asthmatic attacks.

PYRROLAZOTE

Vander Brook et al[349] reported that Pyrrolazote showed the same degree of antihistaminic activity on isolated smooth muscle as did Pyribenzamine, but was active seven times as long. In the usual guinea pig, dog and cat tests, the acute toxicity of Pyrrolazote was about one-half that of Pyribenzamine when given intravenously. Chronic toxicity studies in rats shows that a dose of 10 mg./kg. orally, five days each week for ten weeks, was harmless. Very large doses caused degenerative fatty infiltration of the liver.

The clinical studies by Ogden, Derbes and Cullick[350] showed that patients with severe symptoms noted significant difference in relief, while those on mild or moderate symptoms reacted almost equally to both the drug and the placebo. The patients took the placebo and Pyrrolazote tablets for a week each, alternately, for varying periods of fifteen to twenty weeks, only one week's supply being dispensed, the patient noting on a form each week the time of onset of attacks, the severity of symptoms, the duration of attacks and the number of tablets taken and the frequency with which the tablets were ingested. Sixty-one per cent of forty-six patients suffered from bronchial asthma. The severely affected patients demonstrated 10.55 hours of severe symptoms each week while on the placebo tablets in comparison with 5.08 hours of severe symptoms each week while taking Pyrrolazote. For total symptoms, mild, moderate and severe, there was an average of nineteen hours while taking the placebo, as compared to twelve hours while taking the drug. No patient knew when he was taking a placebo or the drug tablets. The authors note that side reactions are easily subject to false interpretation, since patients are frequently warned of their possibility and, if they have experienced them while on the drug, they are quite apt to complain when taking the placebo. Mild drowsiness was a complaint on thirty occasions with the drug and on twenty-one with the placebo, with severe drowsiness by one patient taking either. Nausea was experienced by thirteen patients on Pyrrolazote, and nine on the placebo. No patient complained of severe nausea while taking the drug, but one of such nausea on the placebo. Vomiting occurred respectively in six patients on Pyrrolazote and four on the placebo. Headache occurred in eighteen and twelve respectively and palpitations in one and none, respectively.

Eight patients had no side reactions with Pyrrolazote and eighteen on the placebo. The authors conclude that Pyrrolazote is a potent antihistaminic agent which compares favorably with other similar preparations. Work is in progress with a two-stage tablet made up with 25 mg. of the drug in the outer coat, with 25 mg. present in an inner ileosol-coated tablet.

THENFADIL (WIN 2848)

Thenfadil is a relative newcomer to the field. The pharmacological properties have been reported by Hoppe et al[351] who show it to be significantly more toxic than structurally similar antihistaminic agents. It would appear that of the bromo and chloro analogues, the first is approximately equal to, and the second less active than, Pyribenzamine. The laboratory studies indicate that the drug itself is approximately twice as active as Pyribenzamine. In a second communication, Hoppe and Lands[352] demonstrate that the average acute toxicity is 10 per cent greater than Pyribenzamine when given intravenously; 30 to 100 per cent greater when given subcutaneously and intraperitoneally in mice; and 30 per cent greater given orally in mice. It is 30 per cent less toxic than Benadryl given orally in mice. The drowsiness in mice was measured by the mean waking time from sleep induced by 100 mg./kg. of Evipal and was discovered to be prolonged 8 per cent by Thenfadil, 11 per cent by Pyribenzamine, and 43 per cent by Benadryl, each given in doses of 10 mg./kg. subcutaneously.

Studies in human subjects by Luduena and Ananenko[353] showed that in twenty-three the wheal induced by histamine (1 to 2 per cent) solution applied to the skin was inhibited or controlled by the application of Thenfadil (5 per cent) to the same area; if given before histamine, the effects were aborted. The clinical evaluation of Thenfadil will probably be available in the near future.

THEPHORIN

The pharmacological properties of Thephorin have been studied by Lehmann[354] and also by Lehmann and Stefko.[355] The usual protection to histamine aerosol was measured, the acute toxicity of Thephorin by various routes of administration being tabulated. Worthy of note, however, is the action of Thephorin on histamine-induced gastric secretion in dogs and on gastric ulcer formation in rats. The anti-ulcer effect of Thephorin is "striking." The evidence available suggests that the effect may be considered as a chemical vagotomy. In the same animals, atropine was ineffective, while Thephorin reduced histamine-induced gastric secretion in dogs with Heidenhain pouches by about 30 per cent. The effects in cats, rabbits, dogs and guinea pigs were later reported upon by Lehmann and his colleagues[356] in a third detailed and well-controlled series of experiments.

The tolerance studies for Thephorin by Boyd et al,[357] using 100 normal subjects varying in age from seventeen to seventy-nine years and given doses of 75 to 600 mg. Thephorin orally daily for at least one week, showed dehydration of the mucous membranes to occur in 22 per cent and insomnia in 21 per cent. In all, forty-two subjects developed one or more toxic symptoms. In five subjects taking 300 mg. daily, the drug had to be stopped because of the severity of the side reactions, but in other subjects taking Thephorin for one month there were no significant changes in the electrocardiogram, the non-protein nitrogen of the blood, the peripheral blood count, or the urine. The drug is generally less toxic, weight for weight in daily doses of 150 to 600 mg., than are Benadryl or Pyribenzamine.

Used topically by Ellis and Bundick[358] a 5 per cent Thephorin ointment in a carbowax base or a 5 per cent lotion for twenty-eight to sixty-eight days in fifty patients gave good results in thirteen, fair in thirteen and poor in twenty-four. In fourteen patients, the eruption became worse and tended to spread, and five of these

patients reacted positively to patch tests with the ointment. Three of five patients reacted to the lotion. The other nine could not be tested because of the aggravated skin lesions. According to Laymon, Madden and Schmid,[359] patch tests on 324 patients with dermatoses who had used Thephorin in the 5 per cent standard ointment for one week to ten months showed a positive patch test to two women with neurodermatitis, and one man and two women with contact dermatitis, pruritus vulvae and atopic dermatitis. A number of reactions to the base were seen, these patients giving negative reactions to the solution.

In 1948, Wooldridge and Joseph [360] reported that neurodermatitis was completely cured in two of twenty-three patients, and more than 50 per cent improved in fifteen patients following therapy with Thephorin. Three patients were not benefited and one became worse. All of the patients took the oral medication, except two who were treated only with the ointment. Some of the patients, who were infants, received 15 to 20 mg. daily, with four infants responding with irritability and sleeplessness. The authors summarized their work saying that seventeen of the twenty-three patients showed more than 50 per cent clearing of the condition, seven over 75 per cent clearing, and eight between 50 and 75 per cent. Two patients were helped slightly, three unchanged and one became worse. Two, as noted above, were cured. In a second report[361] the authors listed nineteen of twenty-three patients suffering from disseminated neurodermatitis relieved and nine of fourteen patients from circumscribed neurodermatitis. The ointment was applied three times daily and massaged into the skin. Of the seventeen patients with circumscribed neurodermatitis, fourteen were treated while three served as controls. Of these fourteen, three had a high initial improvment with a relapse later; five had 100 per cent improvement subjectively but 50 per cent or less objectively, while four had more than 75 per cent improvement subjectively and 50 to 75 per cent objectively, while two patients had less than 50 per cent improvement subjectively and none objectively. The three control patients given the ointment base showed no improvement whatsoever. Side reactions were infrequent and one patient showed a positive reaction to patch tests.

Laymon and Schmid[362] continued their work with Thephorin and reported that pruritus was adequately relieved in 80 per cent of fifty-eight patients with various dermatoses, including circumscribed disseminated neurodermatitis, eczematous eruptions, lichen planus and psoriasis and dermatophytosis, the 5 per cent carbowax ointment being used. In another group of eighteen using the 5 per cent Thephorin lotion, nine of eleven with contact dermatitis and two of three with dried lichenified plaques and all of four with neurodermatitis obtained relief of their pruritus.

In dermatoses characterized by wheal formation, Kesten and Sheard[363] found the ingestion of Thephorin (50 mg.) gave relief to thirty-three of forty-one patients and also stopped the pruritus in twenty-two of thirty-nine with intensely pruritic dermatitis. In twenty-one patients, however, treatment was discontinued because of side effects which in order of frequency included: nausea, anorexia, headache, drowsiness, palpitations, restlessness, depression, vomiting, indigestion, and increase of itching and dizziness.

The most recent report by D'Avanzo[364] proved the contradictory nature of such clinical studies. The author treated seventy-four cases of pruritic dermatoses, twenty-four of whom suffered from disseminated neurodermatitis most of whom did poorly, eight only showing an initial slight-to-fair improvement, with six refractory and two becoming sensitized to the ointment, the greater number of the patients becoming worse after treatment. Of twenty-six patients wih circumscribed neurodermatitis, 39 per cent were very much improved with two becoming refractory; twenty-four additional patients with miscellaneous dermatoses, including a typical case of nummular eczema, improved in one-half of the group, four becoming refractory and two becoming definitely sensitized. The sensitizing index of Thephorin ointment is given at 16 per cent but should read, due to an arithmetical error, 5.4 per cent.

Frank[364a] used Thephorin tablets (25 mg.) and the syrup (10 mg./dram) in 140 allergic patients, who received the tablets one to three times daily, the dose being increased until the patient derived benefit from medication or until side reactions occurred. Best results were obtained in cases of non-seasonal and seasonal rhinitis. Other conditions treated include allergic conjunctivitis, urticaria, angioneurotic edema, neurodermatitis, contact dermatitis, and other miscellaneous manifestations. Side effects occurred in 38 per cent of the 140 subjects studied, but even the most severe reactions required no measures other than discontinuation of the drug. Insomnia was prevented by the concomitant administration of a mild sedative.

Bedford Shelmire[364b] used Thephorin ointment in 305 patients with successful results in 62 per cent of twenty-nine patients with atopic dermatitis, 68 per cent success in fifty-seven patients with contact dermatitis, 91 per cent success in fifty-six patients with circumscribed neurodermatitis, 77 per cent in nine with pruritus ani or vulvae, and 76 per cent relief in miscellaneous pruritic dermatoses. The average successful percentage results were 76.4 for 305 patients.

The miscellaneous uses of Thephorin include the 5 per cent ointment as applied locally to bee stings, or to ant bite areas in eight patients by Strauss,[365] who reports that the intense pain and stinging sensation were relieved within two minutes with no local swelling occurring. Local pin-point areas of petechial hemorrhages disappeared within seventy-two hours.

Schloss[366] noted that of forty-one patients with 126 complaints referable to the digestive tract were relieved of sixty-nine symptoms in twenty-six patients, with twenty-six more partially relieved, Thephorin being administered thirty minutes before eating to those patients who reacted immediately after their meals, and thirty minutes after eating to those who reacted after a longer interval. The best results were obtained in the alleviation of diarrhea, nausea and abdominal pain or fullness. No note is made of ill effects.

A report by Berger[367] showed that Thephorin relieved both the postencephalitic and idiopathic types of Parkinson's disease, doses of 25 to 50 mg. being administered two to four times daily. Six of twenty-four patients had to discontinue treatment because of side reactions, but of the remaining eighteen, thirteen responded favorably. As noted above, Thephorin and also Benadryl and Neohetramine and Pyribenzamine were used by Judd and Henderson[368] in the treatment of primary tuberculosis, doses varying from 50 mg. three times a day initially, to doses of 500 mg. daily, the patients receiving at least ten weeks of treatment. A decrease in the coughing sputum, fever and other symptoms was noted, with a lessening of the skin tests. The best results were obtained against the acute exudative type of tuberculosis, the treatment losing its effectiveness as tuberculous lesions increasd in chronicity.

By including Thephorin with hyposensitization treatment, Maietta[369] discovered that patients could take high pollen dose levels within a short period of time, with a substantial decrease in the number of required injections. The author feels that large pollen doses conferred a "tremendous degree of protection, heretofore not always obtainable to all the patients during their specific season." Thirty-two per cent of the patients reported 100 per cent protection; twelve of fourteen co-seasonally treated patients reported 100 per cent protection, and the remaining two suffered only mild hay fever symptoms. The doses were two to three times those recommended, and side reactions to Thephorin and eight other antihistaminic agents employed were encountered in only twenty-seven patients (60 per cent) but were not sufficiently severe to warrant discontinuation of the drug.

The well-publicized paper by Brewster[370] on Thephorin in the treatment of common colds needs only brief mention. The drug was used in 25 mg. doses at four-hour intervals, for three doses, as the only treatment in a series of 2,614 patients suffering from colds. Parallel cases were given an equal number of placebo tablets. Fifty-four per cent of the cases who were given the drug, and who started treatment within

six hours, were cured in twenty-four hours; and 75 per cent of all cases who started within forty-eight hours were either cured or considered the treatment satisfactory. On the other hand, those who started treatment within six hours with the placebo tablet showed a 32 per cent cure. The side effects, in order of frequency, of those who took the drug were drowsiness in 12 per cent, dizziness in 6 per cent, insomnia in 6 per cent, nervousness in 3 per cent and headache in 3 per cent. Of those who received the placebo drug, 10 per cent complained of side effects. Not only were 32 per cent of the patients who took the placebo cured six hours after symptoms but 21 per cent reported improvement and 47 per cent were unimproved. After twelve hours or less, 28 per cent of the patients taking the placebo were cured, 25 per cent were improved and 47 per cent unimproved. After forty-eight hours, 26 per cent reported cure, with 29 per cent improvement and 45 per cent unimproved.

A clinical report on the use of Thephorin in allergic conditions was made by Reynolds and Horton[371] who treated sixty-two patients with various types of allergic manifestations, reporting that thirty-three obtained excellent relief and twenty-three no relief from daily oral therapy of 25 to 200 mg. Seventeen of twenty-two hay fever patients achieved excellent relief, seven of eleven patients with vasomotor rhinitis, five of six with cold allergy; one of three with acute urticaria and none of three with each, chronic urticaria and migraine. Of the patients with atypical histaminic cephalalgia, two of nine achieved excellent relief as did patients with Raynaud's disease, dermographia, and erythromelalgia. These same patients were either unaffected by, or sensitive to, Benadryl and Pyribenzamine. No serious toxic effects were seen.

In 1948, John Peters[372] reported on 142 patients good results being seen in sixty-six of sixty-eight with hay fever; thirty-one of thirty-four with hay fever and asthma; three of seven with bronchial asthma; all of seven with pollen asthma; eight of ten with dust vasomotor coryza; nine of ten with various types of dermatoses, one patient with sinusitis, and one with hay fever and dermatitis. Fair results were obtained in one of two patients with migraine, but side effects occurred in fifteen patients, the most frequent being gastric disturbances, insomnia, nervousness and excessive perspiration.

Cohen et al[373] used the drug in 292 patients, and reported 75 to 100 per cent relief in forty-two of eighty-three patients with bronchial asthma, 105 of 161 with allergic rhinitis, thirteen of eighteen with angioneurotic edema, five of eighteen with allergic dermatitis and eight of twelve with migraine. Fifty-four patients complained of nausea and vomiting, constipation, palpitations, insomnia, headache and urticaria. Discontinuation of the drug was necessary in eleven. Patients were alternately given Thephorin and Pyribenzamine and preferred the former. Gelfand[374] discovered that the administration of 50 to 100 mg. of the drug improved thirteen of twenty-two cases of bronchial or pollen asthma, twenty-nine of sixty-four with seasonal hay fever, eleven of twenty-nine with perennial allergic rhinitis, five of nine with acute or chronic urticaria, atopic eczema or contact dermatitis, and none of five with angioneurotic edema. Serious side effects were reported as infrequent. In the series studied by Criep and Aaron,[375] the drug brought complete relief to 44 per cent of 180 patients with hay fever, 35 per cent of seventy-one with allergic rhinitis, 16 per cent of seventy-one with bronchial asthma and 12 per cent of eight with contact dermatitis. It substantially helped 43 per cent of thirty cases of urticaria and angioneurotic edema. The side effects were chiefly nervousness and palpitations, nausea and vomiting or insomnia, but also drowsiness, headache, constipation and urinary symptoms occurred in 23 per cent of 389 patients. Pennypacker and Sharpless[376] reported that with 50 mg. doses daily, thirty-one of forty patients with hay fever were helped, twenty-one markedly and ten moderately, with slight improvement in six and no effect in three. Insomnia affected fourteen; tension, nine; dizziness, grogginess,

lassitude, urinary urgency or chilliness, five each, and gastrointestinal disturbances, one. Some patients are reported as finding the stimulation of the drug pleasant.

It should be noted that the last three reports were all published in September of 1948, as were the next three. Sternberg and Gottesman[377] treated seventy-six patients, of whom five experienced insomnia, and one, nausea. Eighteen of forty-one patients with hay fever, nine of twenty-six with bronchial asthma, none of six with vasomotor rhinitis, two of two with urticaria, and none of one with migraine reported good results, with eleven of the seventy-six reporting fair results and the remainder negative effects. The series by McGavack et al[378] on 136 patients showed twelve of sixty-six with hay fever, five of twenty-five with allergic rhinitis, one of twenty-four with bronchial asthma, five of sixteen with urticaria, none of two with contact dermatitis, and one with Fox-Fordyce disease, food allergy and migraine receiving complete relief. Sixty-five patients reported improvement and the remainder no effect. The results are said to be comparable to that of Benadryl in hay fever and urticaria, while the drug was less effective in vasomotor rhinitis and asthma. The toxic symptoms in order of decreasing frequency were: dryness of the mouth, insomnia, constipation, dizziness, jumpiness, burning of the conjunctivae, intestinal cramps, drowsiness, weakness and palpitation, untoward reactions occurring in twenty-two of the patients with hay fever, in seven of those with asthma and in seven with rhinitis. Paul et al[379] reported fair to excellent results in 154 of 197 patients with hay fever, and fifty-four of seventy-one with non-seasonal vasomotor rhinitis. Fair to excellent results occurred in six of twelve patients with bronchial asthma. Of the total number of 280 patients treated, side reactions consisted of sleeplessness, nervousness, chills, nausea, headache and dryness of the mouth and throat occurring in seventy-five patients, with eleven discontinuing treatment because of severe reactions.

In the same year, and to patients undoubtedly exposed to the same type of season, Monchek[380] administered Thephorin to 113 patients for 168 allergic symptoms for one to 171 days. Of those with hay fever, thirty-seven of forty-eight had relief lasting several hours, as did twenty-six of forty-five patients with bronchial asthma; forty-two of sixty-one with vasomotor rhinitis; eleven of twelve with cutaneous allergies, such as acute or chronic urticaria, contact dermatitis and atopic eczema. In thirty-seven patients, the untoward reactions seen included: insomnia, irritability, drowsiness, dizziness, respiratory distress, urinary retention and nausea. Schwartz and Leibowitz[381] reported symptomatic relief in forty-six of sixty patients with hay fever, thirty-three of fifty-five with vasomotor rhinitis, three of twenty with bronchial asthma, three of four with chronic urticaria and one with contact dermatitis. Twelve of the ninety-five patients suffered side reactions, which are listed as drowsiness, dryness of the mouth, headache, indigestion, tiredness, dizziness, palpitations, or bitter taste, none being sufficiently severe to require discontinuation of medication.

In the series studied by Levin and Moss,[382] consisting of 109 allergic children, the drug was found effective in 81 per cent of those suffering from hay fever; 75 per cent of pollen asthma, 73 per cent of perennial asthma, 66 per cent of allergic rhinitis, 75 per cent of infantile eczema and urticaria. Reactions sufficiently severe to require discontinuation of the drug occurred in 8 per cent of the patients, with side reactions in 20 per cent of the group, the drug having a mild sedative effect in children in contrast to the stimulating effect seen in adults. Maietta[383] used the drug intravenously in seventeen patients with bronchial asthma, of whom four were in status asthmaticus. It was noted that the wheeze and dyspnea lessened in one hour with the vital capacity being increased and the patients losing their epinephrine-fastness. Migraine was relieved in two patients within a few minutes and within one hour with intramuscular Thephorin. The results in five patients with angioneurotic edema and urticaria and two with urticaria and one with eczema and angioneurotic edema were striking with the intravenous medication.

TAGATHEN (CHLOROTHEN)

In a preliminary report, Taub, Miller and Taub[384] reported that Tagathen gave three to four hours relief in thirty patients with seasonal and perennial allergic rhinitis and three with urticaria, the usual dose being 100 to 200 mg. daily. An urticaria patient was given 25 mg. every two hours to a top dose of 200 mg. daily. In three patients, untoward reactions, such as drowsiness, vertigo, headache, confusion, weakness, palpitations, diarrhea and abdominal cramps appeared, with severe diarrhea necessitating discontinuation of the drug in one patient, who was also unable to tolerate other ethylenediamine-derived antihistaminic agents. One of the patients was also unable to take Pyribenzamine.

Phillips and Fishbein[385] used Chlorothen to treat ninety-two patients with colds, the tablets (Caubren) also containing acetophenetidin and caffeine, the tablets being taken every three hours for a minimum of forty-eight hours. The average duration of colds in the series was 2.7 days, while in comparison among seventy-four patients given aspirin alone, the average duration of colds was 5.3 days. Two patients discontinued the use of the drug because of light-headedness and dizziness, while ten patients could not use aspirin because of gastrointestinal disturbances and dizziness.

TRIMETON AND CHLOR-TRIMETON

The chemical characteristics, the acute chronic toxicity studies and the antihistaminic action of Trimeton *in vitro* and *in vivo* with mice, rats and dogs, cats and guinea pigs has been the subject of a detailed report by Labelle and Tislow[386] and by Sperber and his colleagues.[387,388] The clinical evaluation by Brown et al[389] on 227 patients presenting twenty allergic and non-allergic syndromes, alone and combined, shows that on 6.25 to 25 mg. one to four times daily, 61 per cent were completely symptom-free and an additional 22 per cent moderately comfortable. On those with hay fever, 90 per cent were relieved, of urticaria, 81 per cent; and with mild extrinsic bronchial asthma, 80 per cent. Side reactions, chiefly drowsiness, appeared in 16 per cent of the patients, being sufficiently mild in 9 per cent to permit the continuation of the drug and severe in only 6 per cent in whom the medication had to be discontinued. Wittich[390] administered 25 mg. three times daily to 125 patients, reporting good and fair results in thirty-one of thirty-three with hay fever; nine of eighteen with perennial allergic rhinitis; ten of thirty-eight with bronchial asthma; nine of thirteen with urticaria; one of one with angioneurotic edema, one of four with gastrointestinal allergy, five of six with atopic dermatitis, none of two with contact dermatitis, two of two with generalized pruritus and six of seven with allergic headaches. Nausea occurred in one patient with perennial allergic rhinitis, recurring when treatment was resumed; one patient developed vertigo and abdominal pain. One person with hay fever who had obtained good relief with the medication subsequently developed bronchial asthma.

Schiller and Lowell[391] found Trimeton effective in forty-seven of fifty-five patients with perennial allergic rhinitis, reporting satisfactory or partial relief; thirteen of fifteen with hay fever and twelve of twelve with urticaria. The relief of symptoms usually occurred within thirty minutes and lasted for more than three hours. The drug failed to relieve one patient with vernal catarrh and one with atopic dermatitis. Drowsiness was seen in six of the patients with dryness of the mouth in three and weakness in one. Goldman[392] treated eighty patients with various allergic syndromes with doses of 12.5 mg. three times daily, or 25 mg. every four hours. Sixty-one of the patients had complete temporary symptomatic relief, with two reporting 50 per cent relief, and no improvement being noted in seventeen. In his series, the drug provided no relief when bronchial asthma was present. Side effects include sleepiness in three patients and insomnia in one.

Allison and Robinson[393] treated thirty-six patients with allergic syndromes with Chlor-Trimeton (2 to 4 mg. three times daily). Relief was reported in three of three

patients with dermographism, one of four adult patients with atopic eczema, two of three children with atopic eczema, six of seven with urticaria, four of five with angioneurotic edema, one of two with bronchial asthma, five of five with seasonal hay fever, two of three with vasomotor rhinitis, one of two with dermatitis venenata, and one case of pruritus vulvae. Only one patient reported an undesirable side effect, namely paresthesia. Vickers and Barrett[394] also showed the drug to possess high therapeutic efficiency and low toxicity. Gaillard[395] administered Chlor-Trimeton to 332 office patients, presenting over 550 symptoms or syndromes, including hay fever accompanied by bronchial asthma, hay fever, asthma due to pollen or infection, infective allergic and other types of vasomotor rhinitis, urticaria and miscellaneous affections, potentially allergic in origin. Groups of eight, 157, 158 and nine were respectively given doses of 1, 2, 4 and 8 mg. three times daily. On the 2 to 4 mg. dose four times daily, twenty-two of twenty-nine patients with pollen asthma achieved good or fair results, as did fifty-four of sixty-six with mixed (allergic and infectious) asthma, nine of forty-six with intrinsic asthma, twenty-one of thirty with vasomotor rhinitis of various origins, seven of ten with urticaria and angioneurotic edema, one of sixteen with eczema and dermatitis, one of one with vertigo due to pollen and one of four with migraine. Thirty-nine per cent of the patients were aware of the effect of the medicine within 15 minutes, with 44 per cent reporting the effect as occurring in 15-30 minutes. Eighty per cent of the patients reported effects within thirty minutes for all dosages. The high and low doses both lasted equally long, from six to twelve hours. Considering all of the cases of hay fever together, 82.8 per cent of those receiving the 2 mg. dose and 77.4 per cent of those receiving the 4 mg. dose achieved improvement. The author concluded that doses no greater than 4 mg. were satisfactory. The incidence of side reactions was 10.8 per cent, the most frequent being slight drowsiness, one patient complaining of vertigo, two patients on 4 mg. doses having gastrointestinal upsets. The author concludes that the drug has an extremely low toxicity and is likely to cause no more than three per cent of severe side reactions.

In summary, it appears that the antihistaminic drugs are suitable only for symptomatic relief. In general, they do not appear to affect the formation of antibodies or the antibody-antigen reaction. When the drugs are discontinued, the symptoms return if the conditions leading to the allergic state have not changed. The antihistaminics are most effective in conditions involving surfaces which have a good vascular supply, including the skin and mucous membranes, and which may reasonably be considered to arise through the mediation of histamine. Thus, urticaria, including that due to drug reactions, and hay fever appear to show the highest incidence of relief in that order. The cough sometimes associated with asthma may be alleviated, although the bronchospasm is not, excepting by intravenous or aerosol medication. Conditions involving deep-seated allergic states, that is, tissues where equilibrium with the humoral system is not as readily attained, are less affected by the antihistaminic drugs. This appears true in those conditions in which "intrinsic" histamine is already within the cells as compared to "extrinsic" histamine which must reach and affect cell surfaces of distant shock organs.

Antihistaminic drugs are also less effective in conditions where histamine may not be the mediator or may be only one of the mediators. Such are not as readily relieved and may not at all be affected. This is not surprising in drugs which have been tailored for their specific activity against histamine. Some drugs have activities other than their antihistaminic action and thus relieve conditions not related to histamine action.

The antihistaminic drugs have found considerable application as pharmacological tools for differentiating between conditions presumably due to histamine and those due to other agents, both in research and in diagnosis. Their reliability in this

respect necessarily depends on their antihistaminic specificity rather than upon the multiple action effects, true of almost all at present available.

In the following list designations of compounds beginning with "F" refer to Fourneau compounds; those with the letters "RP" to products of the laboratories of the Societé des Usines Chimiques Rhône-Poulenc of France. Such compounds have been arranged in the order of the alphabet for ease of tabulation.

In searching the literature, the same compounds may be found under different designations. This is frequently true of the chemical name since some indices or authors may prefer to designate the compounds as derivatives, for example of ethylenediamine, while others may wish to consider them as derivatives of pyridine. Moreover, the usual procedure seems to be to announce the compound first by its chemical name, if its structure is known, or its laboratory number, then when the product appears to have real value, a shortened designation generally a combination of letters and numbers as 3015 RP, followed by a brand name when the compound is ready for commercial use. Literature concerning a given product will contain references in all three types. For this reason, and to simplify ready application of the table, the compounds have been listed under a number of headings, with cross references.

For lack of space, many compounds have not been listed. This includes many of the earlier compounds which were found to be of slight value or were too toxic for human use. An attempt has been made to include the compounds more frequently encountered in the medical literature.

The compounds are listed alphabetically under the identifying number or common or commercial name. Space does not permit listing under the various chemical headings that might be employed.

LIST OF ANTIHISTAMINIC PREPARATIONS AVAILABLE (JANUARY 1, 1950)

A 446 see Linadryl

A 524 see Benadryl
AH 42 see Thenylene
Amidryl see Benadryl (A/S Medicinalco, Copenhagen (tablets 0.5 gm.)

Anahist (Anahist Company, affiliate of Nepera Chemical Company)
active ingredient Neohetramine (25 mg.)

Antamine (Grove Laboratories)
pyranisamine maleate (25 mg.)

Antastan (Ciba), see Antistine

Antazoline, see Antistine

Antergan RP2339 (Rhone Poulenc), Lergitin; Dimetina; B 97; Bridal
N'phenyl-N'benzyl-N-dimethyl-ethylenediamine
N-dimethylaminoethyl-N-benzylaniline
N-B-dimethylaminoethyl-N-phenylbenzylamine
(tablets 0.1 gm.; ampoules 0.05 gm.)

Anthallan (Medico Chemical Corporation of America)
lactone of B-gallic acid ethanol-2-di(n-butylamine)
3'di(n-butyl)aminoethyl-4,5,6-trihydroxybenzo(1,2c)furan-1'(3') one

Anthisan (May and Baker); Neoantergan; RP 2786; see under Neo-Antergan
p-methoxy benzyl-pyridyl-dimethyl-ethylene diamine

Antistin (a,e) (Ciba); Antastan; phenazoline hydrochloride, M 5512
2 (phenylbenzylaminomethyl) imidazoline
(2-methyl-2-imidazoline) benzylphenylamine
2-(N-benzyl-anilinomethyl)2-imidazoline
Scored tablets 100 mg.; ophthalmic solution 0.5% pH 6.94; Nasal solution 0.5%, pH 6.2

B97, see Antergan

B 194
N,N,dimethyl,N'-2-thiazolyl,N'-p-methoxybenzylethylenediamine hydrochloride

Benadryl (Parke Davis); Amidryl; A 524; S 51; diphenhydramine HCl, see also Hydryllin
B-dimethylaminoethyl-benzhydryl ether HCl
B-benzohydryloxy, N,N,dimethylethylamine HCl
(Capsules 25 and 50 mg.; Elixir 10 mg. per 4 c.c.; parenteral solution 10 mg. per c.c.; cream 2%)

Benylin Expectorant
Benadryl hydrochloride, ammonium chloride, sodium citrate, chloroform, menthol, raspberry flavored syrup

Bridal, see Antergan

Bromobenzyl DPE (Stamford Research Lab)
N,N,dimethyl,N'(p-bromobenzyl)N'-(2-pyridyl)ethylenediamine hydrochloride

Bromothen (Stamford Research Lab); Histagon B (Lederle)
N,N,dimethyl-N'-(2-pyridyl)-N'(5-bromo-2-thenyl)ethylenediamine

C 5581H (Bristol) 01500 (Lilly)
o-benzylphenyl-betadimethylaminoethylether

Caubren compound (Whittier)
(Chlorothen 25 mg.; acetophenetidin 320 mg.; caffeine 32 mg.)

Chlorcyclizine, Perazil (Burroughs Wellcome); Compound 47-282; Di-Paralene (Abbott)
1-(4-chlorobenzohydryl) 4-methylpiperazine 2HCl

C(S), 63 see Pyribenzamine

Chlorothen (Whitties Lab) Histagon C (Lederle); chlorothen citrate chemically the same as Tagathen (Lederle), see also Caubren compound
N,N,dimethyl-N'-(2-pyridyl)-N'-(5-chloro-2-thenyl)ethylenediamine
2-[(5-chloro-2-thenyl) (B-dimethylaminoethylamino)]pyridine
N'pyridyl-N'-5-chlorothenyl,N-dimethyl-ethylenediamine
Tablets 25 mg.

Chlorprophenpyridamine, see Chlortrimeton

Chlortrimeton (Schering); chlorprophenpyridamine maleate, see also Coricidin
1-(p-chlorophenyl)-1-(2-pyridyl)3-N,N,-dimethylpropylamine maleate
1-(p-chlorophenyl)-1-(2-pyridyl)3-dimethylaminopropane maleate
Scored tablets 4 mg., 2 mg.

Compound 1695, see Searle 1695

Compound 0-2315
dimethylaminoethylphenyl-alphathienyl glycolic acid ester
alpha phenyl ester of thiopheneglycolic acid

Compound 47-282 Chlorcyclizine; see Perazil

Coricidin (Schering)
(Chlortrimeton 2.0 mg., acetylsalicylic acid 3.5 gr., acetophenetidin 2.5 gr., caffeine 0.5 gr.)

CS 63, see Pyribenzamine

Decapryn succinate (Merrell) Doxylamine
2-dimethylaminoethoxyphenylmethyl-2-picoline
2-[alpha-(B-dimethylaminoethoxy)alpha-methylbenzyl]pyridine
alpha (2-pyridyl-alpha-phenyl)-B-dimethylaminoethyl ether
(Scored tablets 12.5 and 25 mg.; syrup 6.25 mg. per 5 c.c.)

Diatrin (Warner) W 50; RP2740
N,N dimethyl,-N'phenyl,-N'(2-thienylmethyl)ethylenediamine monohydrochloride
N,N,-dimethyl,N'-(2-thenyl)-N'-phenyl-ethylene diamine
(Tablets 50 mg.)

Dimenhydrinate, see Dramamine

Dimetina (Lepetit S. A. Milan), see Antergan

Di-Paralene (Abbott), see Chlorcyclizine
N-(4-chlorobenzohydryl)N'methyl piperazine HCl

Diparcol (May and Baker, England) RP 2987
10(B-diethylaminoethyl)phenothiazine HCl

Diphenhydramine, see Benadryl

Doxylamine succinate, see Decapryn

Dramamine (Searle) dimenhydrinate
Compound of B-benzohydryloxy-N, N-dimethylethylamine and 8-chlorotheophylline; B-dimethylaminoethylbenzohydryl ether 8-chlorotheophyllinate
diphenhydramine compound with 8-chlorotheophyllin
(Tablets 100 mg.)

Hetramine (RP 2971)
N,N-dimethyl,N'-benzyl-N'(alpha pyrimidyl)ethylenediamine
2[benzyl (2-dimethylaminoethylamino)]pyrimidine

Histadyl (Lilly) Thenylpyramine, Thenylene (Abbott); Meth(h)apyrilene, AH 42; Lilly 01013
N'-pyridyl-N'-thenyl-N-dimethyl-ethylenediamine HCl
(Capsules 25, 50, 100 mg.; coated tablets, 50 mg., syrup, 4 mg./c.c; parenteral solution, 20 mg./c.c.; cream, 2%; ophthalmic ointment, 0.5%)

Histagon B (Lederle), see Bromothen

Histagon C (Lederle), see chlorothen

Histaphene (Union Chimique Belge, Brussels)
p-methoxy-benzhydryl-dimethylaminoether HCl
N,N-dimethyl-beta(4-methoxy-benzohydryloxy)ethylamine

Histostab (Boots Pure Drug Co., England)
2-phenylbenzylamino methyl imidazole methane sulfonate

Hydryllin (Searle)
(Diphenhydramin 25 mg.; aminophylline 100 mg.)
Benadryl and aminophylline

Hydryllin with racephedrine, 25 mg.

Inhiston (Union Pharmacal Co.)
Trimeton 10 mg.
1-phenyl-1-(2-pyridyl)-3-dimethylaminopropane

Kriptin (Whitehall Pharmacal Co., American Home Products)
Pyranisamine maleate (Neo-Antergan) (25 mg.)

Lergitin (Recip, Stockholm), see Antergan

Linadryl; A 446
beta-morpholino ethylbenzhydrylether

M 5512, see Antistine

Mepyramine maleate, see Neo-Antergan

Met(h)apyrilene, see Thenylene, Histadyl

Neo-Antergan (Merck) RP 2786; Anthisan; Pyranisamine; Antamine; Kriptin
N-p-methoxybenzyl-N'N'-dimethyl-N-alpha pyridylethylenediamine maleate
N-dimethylaminoethyl-N-p-methoxyamino pyridine
2-(Beta-dimethylaminoethyl)p-methoxybenzylamino pyridine maleate
(Tablets 25, 50 mg.)

Neohetramine (Nepera, Wyeth) Anahist; Thonzylamine, NH 188
N,N-dimethyl-N'(p-methoxybenzyl)N'-2-pyrimidyl)-ethylenediamine HCl
(Tablets 25, 50, 100 mg., Syrup 6.15 mg./c.c.; Cream, 2%)

Nethaphyl (Merrell)
Combination of nethamine and betaphyllamine

Nethapryn syrup (Merrell)
Nethamine HCl 25 mg., theophylline 50 mg., Decapryn succinate 6 mg.

NH 188, see Neohetramine

NU 1504, see Thephorin

Orthoxicol (Upjohn)
(1 c.c. contains dihydrocodeinone bitartrate 36.5 mg., orthoxine, HCl 338 mg., hyoscamine HBr, 2.0 mg., sodium citrate, 6.5 gr.)

Orthoxine
beta (o-methoxyphenyl)-N-methyliopropylamine HCl
alpha, N-dimethyl-o-methoxyphenethylamine HCl

Pentryl (Maltine Company)
methapyrilene HCl 50 mg., ephedrine HCl 16 mg., phenobarbital sodium 16 mg., with and without enteric-coating

Perazil (Burroughs Wellcome); Chlorcyclizine; Compound 47-282, Di-Paralene (Abbott, 1HCl)
1-(4-chlorobenzhydryl)4-methyl piperazine 2 HCl
N-methyl, N'-(4-chlorobenzhydryl) piperazine 2 HCl
Capsules 50 mg.

Phenazoline HCl, see Antistine

Phenergan (Merck Laboratories, Poulenc Frères de Canada, Montreal)
RP 3277; promethazine, Vallergine (May & Baker, England)
N-dimethylaminopropyl phenothiazine
10(2-dimethylamino isopropylphenothiazine
dimethyl amino-2-propyl-1-thiodiphenyl amine
Special coated tablets, 25 mg.

Phenindamine, NU 1504, see Thephorin

Pinex Antihistamine Tablets

Promethazine, see Phenergan

Prophenpyramine, see Trimeton

Prophenpyridamine, see Trimeton

Pyranisamine, see Neo-Antergan

Pyrathiazine, see Pyrrolazoate

Pyribenzamine (Ciba) CS 63, RP 2750 Tripelennamine HCl, Phz
betadimethylaminoethyl-2-pyridylbenzylammonium chloride
N,N-dimethyl-N'benzyl-N' (alphapyridyl)ethylenediamine
2[benzyl(2-dimethylaminoethyl)]aminopyridine
Scored tablets 50 mg.; Elixir 7.5 mg./c.c.; ointment and cream, 2%, nasal solution, 0.5%

Pyribenzamine expectorant with ephedrine (Ciba)
Each 4 c.c. contains pyribenzamine citrate 30 mg., ephedrine sulfate 10 mg., ammonium chloride 80 mg.

Pyrrolazote Abergic (Upjohn)Pyrathiazine; 1 WBR 86
(beta pyrrolidinoethyl) phenothiazine
betapyrrolidine ethylphenothiazine HCl
Tablets 25 and 50 mg.

Resistab (Bristol Myers)
Thonzylamine (Neohetramine) 25 mg.

RP 2339, see Antergan

RP 2740, see Diatrin

RP 2750, see Pyribenzamine

RP 2786, see Neo-Antergan

RP 2971, see Hetramine

RP 2987, see Diparcol

RP 3015 Searle 1627
10 (betadimethylaminoethyl)phenothiazine; dimethylaminoethylthiodiphenylamine

RP 3277, see Phenergan

RP 3356
10-betadiethylaminopropyl phenothiazine

S 51, see Benadryl

S 108 Schering, see Trimeton

SC 1923 (Searle)
N-hydroxyethylmethylaminoethyl)phenothiazine

Searle 1627, see RP 3015

Searle 1695
8-chlorotheophylline salt of 10 (betadimethylaminoethyl)phenothiazine (RP 3015)

Serial 01013 (Lilly), see Histadyl

Spofa III
1-(betabenzohydryloxyethyl piperidine)
Tabcin (Miles) Thenyl pyramine hydrochloride 30 mg. acetophenetidin 0.2 gm. caffeine 0.03 gm.

Tagathen, see chlorothen
chlorothen citrate

Thenefren (Abbott)
Thenylene hydrochloride 50 mg., ephedrine hydrochloride 25 mg.

Thenfadil (Sterling-Winthrop) WIN 2848
N, N-dimethyl N'(3-thenyl)N'(2-pyridyl)ethylenediamine
2[(B-dimethylaminoethyl)(3-thenyl) amino]pyridine

Thenyl DPE (Stamford Research Laboratories) Thenylene, Histadyl
N,N-dimethyl-N'(2-pyridyl)-N'(2-thenyl)ethylenediamine hydrochloride

Thenylene (Abbott) AH 42, Serial 0103 (Lilly); Histadyl; W 53 (Warner)
Metapyrilene; Thenyl DPE, Thenylpryramine
N,N-dimethyl-N'(alphapyridyl)-N'-(2-methylthienyl)ethylenediamine
N'-pyridyl-N'thenyl-N-dimethylethylenediamine
2[(2-dimethylaminoethyl)-2-thenylamino]pyridine
(Tablets 25, 50, 100 mg.; cream, 2%)

Thenylpyramine, see thenylene

Thephorin (Hoffmann-LaRoche) NU 1504; phenindamine acid tartrate
2 methyl-9-phenyl-2,3,4,9-tetrahydro-1-pyridindene hydrogen tartrate
Tablets 25 mg., syrup 10 mg./4 c.c.; ointment, 5%; lotion, 5%)

Thephorin AC (Hoffmann-LaRoche)
Thephorin acetylsalicylic, acetophenetidin, caffeine

Thonzylamine hydrochloride, see Neohetramine

Tostramine
B-thymoxyethyl dimethylamine

Trimeton (Schering) Prophenpyridamine; S108, see also Inhiston
1-phenyl-1-(2-pyridyl)-3-dimethylaminopropane
2-[alpha(dimethylaminoethyl)benzyl]pyridine
Scored tablets, 25 mg., Elixir, 7.5 mg./4 c.c., Cream, 3%

Tripel-enn-amine, see Pyribenzamine

Vallergine (May & Baker, England), see Phenergan

W 50, see Diatrin

W 53 (Warner), see Thenylene

1WBR 86, see Pyrrolazote

Win 2848, see Thenfadil

47-83 (Wellcome Research Lab)
1-benzohydryl-4-methyl-piperazine

75 Bay State Road (Dr. Brown)

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SKIN REACTIONS

(Continued from Page 551)

with each organism? Or is there a common antigen or even irritant in all of them? These questions cannot be answered with finality at present. The fact that children and adults react so regularly to all of these bacteria is quite different from the clear-cut specificity noted with such substances as tuberculin, coccidioidin, and histoplasmin. It is obvious that the delayed reactions noted with the common bacteria cannot be accepted as specific without further evidence.

SUMMARY

1. Tuberculous patients were tested with immunogens and bacterial residues of common bacteria.

2. No specific immediate type reactions were noted with either material.

3. Delayed reactions occurred with both materials on all the children and adults tested.

4. The regularity with which such delayed reactions occur suggests the probability that they are due to a common antigen or irritant.

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News Items

ASSOCIATION OF ALLERGISTS OF SOUTH SWEDEN

On May 6, 1950, the Association of Allergists of South Sweden met at the auditorium of the Radiology Clinic, Hospital Building, Lund, Sweden. After the business meeting the following program was presented: "Rh-isoimmunization" by Dr. B. Broman, Stockholm; "Observations Made During Tests to Develop and Suppress, Intentionally, the Production of Anti-Rh-agglutinations" by Dr. Rune Grubb; and "The Significance of Rh Research for the Science of Allergy" by Dr. Paul Kallós.

PERMANENT COMMITTEE ON PEDIATRIC ALLERGY

At a recent meeting of the Board of Regents at St. Louis, a committee on pediatric allergy was established. Dr. Bret Ratner of New York was invited to become chairman of this committee, with power to appoint the membership of the committee. The new members are Dr. William P. Buffum, Dr. Norman W. Clein, Dr. Susan C. Dees, Dr. Jerome Glaser, Dr. Edward Scott O'Keefe, Dr. Meryl M. Fenton, Dr. Albert V. Stoesser, and Dr. Bret Ratner, Chairman.

COMMITTEE ON PSYCHOSOMATIC ALLERGY

The Board of Regents unanimously decided that a Committee on Psychosomatic Allergy be appointed by President Mitchell and Dr. H. A. Abramson, to work with Dr. Stoesser in setting up a group of papers for one morning session at the next annual meeting. The committee is as follows: Dr. D. Baruch, Dr. Hal M. Davison, Dr. Loraine O. Dutton, Dr. Bennett Kraft, Dr. Hyman Miller, Dr. John H. Mitchell, Dr. Murray M. Peshkin, Dr. Boen Swinney, and Dr. H. A. Abramson, chairman.

BRITISH ASSOCIATION OF ALLERGISTS

The ninth general meeting of the British Association of Allergists was held on July 8, 1950, in the Sir William Dunn School of Pathology, South Parks Road, Oxford, England. After the Council meeting, Sir Howard Florey, F.R.S., Professor of Pathology, University of Oxford, acted as chairman of the morning session. Dr. A. W. Frankland spoke on "The First European Congress of Allergy." Dr. Homer Prince of Houston, Texas, presented his paper, "Histamine Therapy," written in collaboration with Dr. Richard L. Etter; discussion by Dr. Blair Macaulay of Liverpool followed.

The afternoon session was presided over by Dr. R. L. Vollum, director of Public Health Laboratory, Oxford, and bacteriologist to the United Oxford Hospitals. Dr. Boen Swinney of San Antonio, Texas, read "Viral Allergy," followed by a general discussion by members and visitors. Dr. Ethan Allan Brown of Boston, Massachusetts, read "Present Status of ACTH Therapy in the Allergic Diseases," with discussion opened by Dr. Peter Bishop, endrocrinologist at Guy's Hospital, London.

Dr. Vera B. Walker is president of the Council; Dr. A. W. Frankland is secretary.

COURSE IN ALLERGY FOR CLINICIANS

The University of Illinois Allergy Unit, Colleges of Medicine and Pharmacy, Chicago, announces a course in allergy for clinicians to be held from October, 1950, to October, 1951. The curriculum is divided into six sections: Allergy, Medicine, Immunology, Botany and Mycology, Basic Sciences, and Specialties. The course is accredited for one year towards the formal training requirements of either the American Board of Internal Medicine or of the American Board of Syphilology, and is approved for training under the GI Bill of Rights. Enrollment is limited to six students. The fee is $300 for Illinois residents and $600 for nonresidents, or by GI contract.

Courses will be given in Sensitization Mechanisms, Respiratory Allergy, Allergy Clinic, Food Allergy, Preparation of Allergens, Internal Medicine, The Acute Infectious Diseases, Electrocardiography, Psychosomatic Medicine, Immunochemistry, Immunology, Botany, Mycology, Pharmacology, Physiology, Pathology, General Dermatology, Otolaryngology, and shorter courses on Endocrinology, Hematology, Ophthalmology, and Statistics.

Further information may be obtained from Ben Z. Rappaport, M.D., Allergy Unit, University of Illinois, College of Medicine, 1853 West Polk Street, Chicago 12, Illinois.

CANADIAN SOCIETY FOR THE STUDY OF ALLERGY

At Victoria General Hospital Auditorium, Halifax, Nova Scotia, the annual meeting of the Canadian Society for the Study of Allergy was held on June 20. The morning session featured the following papers: "Dermatological Allergy" by Dr. K. A. Baird, West St. John, N. B.; "Dandelion as a Cause of Hay Fever" by Dr. H. C. Jamieson, Edmonton, Alta.; "Antibody Studies in Hay Fever in Children" by Drs. S. Pedvis and H. Bacal, Montreal, Que.; "Some Clinical Problems in Allergy" by Dr. I. H. Erb, Toronto.

At the afternoon session the guest speaker was Dr. Kingsley Johnston, Department of Allergy, Cleveland Clinic, Cleveland, Ohio, who presented "Progress in Allergy During the Past Decade." Other papers read were "The Use of ACTH and Cortisone in Diseases of Hypersensitivity" by Dr. Bram Rose, Montreal, Que.; "The Effects of Phenergan in Experimental Allergic Glomerulonephritis in Rabbits" by Dr. J. Fitzgerald, Toronto, and Dr. J. Hamilton, Queen's University; and "Pneumothorax Occurring in Asthma." A special feature was a film, "Allergy, Immunology—Diagnosis and Treatment" directed by Dr. Leo Criep, Associate Professor of Medicine, University of Pittsburgh.

NEW FEATURE IN THE QUARTERLY REVIEW

The *Quarterly Review of Allergy and Applied Immunology*, published under the auspices of The American College of Allergists, announces a new feature. Arrangements have been made with Dr. Jonathan Forman to add the "Bibliography," formerly appearing in The Letters of The International Correspondence Society of Allergists, to the *Quarterly*. Dr. Forman will be reference editor of this new department. It will include about thirty-five pages in the four issues, averaging twenty-eight references to the page. This valuable bibliography will make the *Quarterly* one of the most complete reference journals in existence today on the subject of allergy.

During the past year the *Quarterly* has published over 2,000 reviews on all phases of allergy and immunology, as well as a comprehensive index to this material. Since the *Quarterly* can be bound by the publisher at a very reasonable price, the references are made readily available and up to date.

Special articles featuring broad and comprehensive studies of particular phases of allergy will appear in each issue. Negotiations are now under way to secure outstanding special articles for next year.

The subscription rate of the *Quarterly* is $7.00 a year to subscribers to the ANNALS OF ALLERGY, $8.00 to nonsubscribers ($1.50 additional postage for foreign countries). Back issues may be obtained at $2.50 an issue from Bruce Publishing Company, 2642 University Avenue, St. Paul 4, Minnesota.

BRAZILIAN SOCIETY OF ALLERGY

The Brazilian Society of Allergy met May 24 in the Noble Hall of the General Polyclinic of Rio de Janeiro, with Dr. Ivolino de Vasconcellos presiding. The following program was presented:

"Historical Panorama of Otorhinolaryngology" by Dr. Antonio R. C. Monteiro.
"The Life and Work of Santorini" by Dr. Erich Gruen.
"Beginnings of Medical Teaching in Brazil" by Dr. Ordival Gomes.
"History of the Leukemias and Pseudoleukemias" by Jayme de Mendonca Castro.
"Vital Brazil-Pioneer in Fight Against Snake Bite" by Dr. Ivolino de Vasconcellos.

ALLERGY COURSES IN HAVANA

The University of Havana, Cuba, is holding its Tenth Session of Courses on Specialties of Medicine, July 10 to August 19. Courses on the allergic diseases are conducted by Dr. José M. Quintero Fossas, Director of the Department of Allergy of the National Council for Tuberculosis, who is a Fellow of The American College of Allergists. These courses serve as refresher courses and for postgraduate instruction.

CONNECTICUT ALLERGY SOCIETY

The annual meeting of the Connecticut Allergy Society was held with the Connecticut State Medical Society on May 3 in Waterbury. New officers are Dr. Barnett P. Freedman of New Haven, President; Dr. Vincent P. Cenci of Hartford, Vice-president; and Dr. Paul Winer of New Haven, Secretary-treasurer.

ARIZONA SOCIETY OF ALLERGY

Another new allergy society is the Arizona Society of Allergy, organized May 2 at a meeting of the Arizona Medical Association. Officers are Dr. William B. Steen, Tucson, President; Dr. Eugene A. Gatterdam, Phoenix, Vice-President.

PERSONAL ITEMS—ACA MEMBERS

M. Coleman Harris, M.D., F.A.C.A., announces an additional office at Suite 400, Professional Building, Los Angeles 17, California. His original office is at 416 North Bedford Drive, Beverly Hills. Dr. Harris' practice is limited to allergy.

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Ellis April, M.D., F.A.C.A., 1530 16th Street NW, Washington, D. C., has recently been appointed Assistant Professor of Clinical Medicine (Lecturer in Allergy) at the Georgetown University School of Medicine.

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It has recently been announced that the Cuban Allergy Society has honored Clarence Bernstein, M.D., F.A.C.A., of Orlando, Florida, with membership in that group.

BOOK REVIEWS

THE NOSE. By Thomas H. Holmes, M.D., Research Fellow in Medicine; Helen Goodell, B.S., Research Fellow in Medicine; Stewart Wolf, M.D., Associate Professor of Medicine; Harold G. Wolff, M.D., Professor of Neurology; all of Cornell University Medical College, New York. With a Foreword by Warfield T. Longcope, M.D., Professor Emeritus of Medicine, The Johns Hopkins Medical School, Baltimore. 154 pages, 37 figures; frontispiece in colors. Price $4.50. Springfield, Ill.: Charles C Thomas, Publisher, 1950.

This monograph consists of extremely interesting and unusual direct observations of a qualitative and quantitative nature, revealing that everyday experiences may give rise not only to emotional responses but to significant physiological changes. The authors have demonstrated that the nasal mucous membranes may either shrink or become engorged, producing the well-known "stuffy" or "runny" nose in reactive individuals in situations of conflict as well as in response to differing chemical and physiological stimuli. The authors interpret these as appropriate protective reaction patterns, which illuminate the correlation of emotional disturbances and respiratory illness.

There are thirteen chapters, most of which are followed by a summary and a bibliography. The relation of hay fever and asthma to the intensely hyperfunctioning mucous membrane is demonstrated by figures and case reports. These "life charts" are very interesting and indicate the extreme accuracy and care taken when observing these reactions.

This is not a text on psychiatry, as the psychiatrists, although given credit for interesting themselves in disturbances of the nose with problems of personality adjustment, are criticized because their observations are made "usually without adequate inquiry into the nature and physiologic mechanisms of the bodily changes." The authors attempt to study the man and his nose as a unit, integrating these points of view for the better understanding of the human organism.

The book should be of interest to all who are concerned with disorders and diseases of the respiratory passages and their relationship to other structures in the head as well as to the more distant organs. As usual with Thomas publications, the paper stock is excellent, the printing and the figures very clear. All allergists will profit by reading this unusual monograph.

THE MERCK MANUAL OF DIAGNOSIS AND THERAPY. A Source of Ready Reference for the Physician. 8th ed. Price $4.50; Thumb-indexed $5.00. Rahway, N. J.: Merck & Co., Inc., 1950.

The appearance of the eighth edition of this all-inclusive manual is most welcome, and being the new "Golden Anniversary Edition" of the Merck Manual, it exceeds all previous editions in scope. Since the beginning of preparation of the new volume in 1946 by the Merck Medical Division, more than 100 clinicians throughout the United States have served as authors or consultants. The editorial board consisted of the editor and four other physician members, aided by seven assistant editors who also were physicians. Continuing revisions to include new developments were made right up to the publication date, May 1, 1950. This first printing of 75,000 copies was exhausted by orders prior to publication; a second printing is now on the press and will be available July 30. This is the best testimony of its use among physicians.

Approximately 1,600 pages in length, the new edition contains 338 chapters in Part I on the diagnosis and treatment of diseases, 82 more chapters than in the preceding edition. New or expanded chapters include those on nutritional deficiencies; radiation reactions and injuries, including those due to atomic bombs; allergies and antihistamines; psychoneuroses; drug addiction; dental emergencies the physician may have to treat; prenatal and postnatal care; and the care of pre-

mature infants. More than 1,175 prescriptions are included, conveniently arranged in categories according to therapeutic action.

All diseases are listed alphabetically, making any subject readily accessible. The section on allergy takes in twenty-nine pages and consists of General Considerations, Allergic Rhinitis, Hay Fever, Perennial Rhinitis, Allergic Conjunctivitis, Bronchial Asthma, Gastrointestinal Allergy, Urticaria and Angioneurotic Edema, Serum Sickness, Physical Allergy, and Prescriptions.

Part II contains new chapters on routine immunization measures, clinical and bedside procedures, laboratory tests practicable for the physician's office, suggested items for the physician's bag, an outline of preoperative and postoperative care, a section on diets, and helpful ready reference data and conversion tables.

The medical experience gained in World War II and the phenomenal advances in medical science since then are reflected throughout the new volume. Some of the other highlights are a complete up-to-the-minute presentation of antibiotic therapy, crystalline Vitamin B-12, Cortisone, and ACTH. Directly following the Foreword there is "Suggestions for Readers," a very helpful page pointing out special subjects in which there may be particular interest. Many of the chapters contain helpful tables; those on Cardiac Arrhythmias and Cardiac Blocks are illustrated by ECG tracings; and a useful miscellany will be found at the end of Part II. This includes recommended daily dietary allowances for men, women, and children; the minimum daily basic requirement of nutrients; various diets used in disease states, with sample menus; food values; food equivalents; office laboratory procedures; alternative proprietary preparations listed as a therapeutic index; ready reference guides of calculation of dosages for infants, children, and aged patients; a table of weights, measures, and equivalents; and an index with headings printed in boldface type.

The book is printed on thin India paper stock in very readable type and bound in a beautiful dark blue durable buckram, which makes it one of the most valuable encyclopedic reference manuals on the physician's desk.